THE URINE
THE GASTRIC CONTENTS
THE COMMON POISONS
and
THE MILK

J. W. Holland, M. D.

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THE URINE

AND

CLINICAL CHEMISTRY OF THE GASTRIC CONTENTS, THE COMMON POISONS, AND MILK

BY

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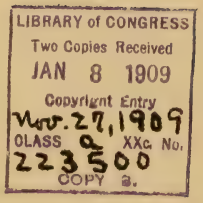
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COMPOSITION

OF

HEALTHY URINE.

Healthy urine is a clear yellow fluid, slightly acid in reaction and holds in solution variable amounts of substances, organic and inorganic.

Average Composition of Normal Urine	Percentage Composition.	Grains per Diem.
Water	96.0	50 fl. ozs.
Solids, as tabulated below	4.0	1000 grs.
Urea	2.000	500
Uric acid	0.040	10
Hippuric acid	0.075	15
Creatinin	0.075	15
Pigment, mucus, xanthin, other extractives, etc	1.000	170
Chlorids of potassium and sodium	1.000	170
Sulphates of potassium and calcium	0.110	40
Phosphates of potassium and sodium	0.120	45
Phosphates of magnesium and calcium	0.080	30

Besides these, there have been found traces of indican, phenol and other aromatic sulphates, diastase, oxalic, and lactic acids, unoxidized sulphur, and phosphorus.

REAGENTS AND APPARATUS FOR URINALYSIS.

Acid, nitric.
" sulphuric.
" hydrochloric.
" acetic. (10 per cent.)
" picric, 5 gr. to ℥j.
Ammon. hydroxid, 20°.
Potass. " (normal solution.)
Silver nitrate (standard solution.)

Buret with pinch-cock.
Pipet, 5 c.c.
Capsule, 50 c.c.
Graduated cylinder, 50 c.c.
Urinometer.
Ureameter. Doremus-Hinds.
Urinary pipet.
Glass rod.

Copper sulphate. (1 in 10 solution.)
Bismuth subnitrate powder.
Potassium chromate, neutral. (1 in 10).
Magnesium mixture ($MgSO_4$, 10; NH_4Cl, 10; aq. ammon. fort., 10; water, 80).
Sodium hypochlorite (Labarraque).
Sodium hydroxid (decinormal).
Alcoholic sol. phenolphthalein, 1 per cent.
Ferric chlorid (1 in 10 solution).
Barium chlorid (standard solution).
Fehling's solution.
Glycerin
Litmus test papers.

Blowpipe.
Watch-glasses.
Wire gauze.
Platinum foil.
Funnel.
Filter paper.
Tripod.
Bunsen's burner.

ARTIFICIAL MORBID URINES.

Artificial urines, for practicing the various tests, can be made up in bulk and kept in stock as follows:

Albuminuria: The white of one egg shaken in a bottle with 100 c.c. (4 fluidounces) of a 2 per cent. aqueous solution of sodium chlorid water filtered and preserved by adding half a drachm of chloroform. For testing, add 5 drops to 100 c.c. of urine.

Albumosuria: Dissolve in urine some Witte's dry peptone.

Glucosuria: Ordinary syrup of the grocers is made of glucose and may be added to urine as required.

Biluria: No satisfactory preparation can be made. The best is ox-gall obtained from the butcher.

Acetonuria: Add a small amount of acetone at the time of testing.

Diacetic Acid: Dissolve in urine, by stirring or shaking, a few drops of ethyl aceto-acetate.

Hematuria: Add fresh blood or blood kept in glycerin or use a scale preparation made by drying blood on glass plates. When wanted the scales are ground in water, filtered and added to urine.

Indicanuria: Add 5 drops of chloroform to the ounce of horse's urine, which is rich in indican. A small amount only must be added to give the reaction to human urine.

Conjugate sulphates: Add potassium ethyl sulphate to normal urine.

Phosphaturia: To make a urine which becomes turbid on boiling, shake with it some freshly precipitated calcium carbonate (made by adding sodium carbonate to calcium chlorid in solution). Filter off the excess.

Chyluria: Add milk to normal urine.

Urobilin: Make an alcoholic extract of fresh human feces by stirring them with strong alcohol and filtering. It retains fecal odor but keeps for several months. For testing add a sufficient quantity of this extract to the urine. The alcohol causes turbidity which may be ignored.

Diazo-compound: Make a solution of 0.1 gm. alpha-amido-naphthalene in 10 c.c. of water and 5 c.c. of hydrochloric acid. To get Ehrlich's *diazo-reaction* add about 1 c.c. of this to 10 c.c. of water or urine.

EXAMINATION

OF

MORBID URINE.

PLAN FOR CLINICAL STUDY.

First. Ascertain from patient the total daily discharge.

Second. Note if the color and odor are normal. If deep yellow, green, or brown, test for biliary pigment (§ 2). If red or chocolate, test for hemoglobin (§ 73, 74).

Third. Determine the reaction with litmus paper (§ 7).

Fourth. Take the specific gravity and calculate the solid urine (§ 5, 6).

Fifth. Having allowed the urine to settle, carefully decant the clear part into a test-tube until it is half full, and examine for albumin by boiling the upper part. One drop of acetic acid must be added if the reaction is not acid. If the flakes formed are white, pure albumin is present. If greenish, probably bile pigment is the cause (§ 2). If red-brown, there is probably blood (§ 73). After twenty-four hours, note the height of albuminous layer. Confirm by test with cold nitric acid (§ 59).

Sixth. Test for glucose by Fehling's and by Böttger's method (§ 37, 45). Determine the amount.

Seventh. Estimate if the chlorids are absent or much reduced (§ 9). Determine the amount.

Eighth. Estimate the amount of urea.

Ninth. Note the naked-eye characters of the deposit, and also the microscopic appearances (§ 83). If amorphous, and the urine is acid, then the deposit is composed of mixed urates; if the reaction is alkaline, then the deposit is phosphatic. To make sure, test for urates and phosphates (§ 83).

THE SAMPLE.

In casual examination for the presence of abnormal constituents, the sample of urine may be voided at any hour of the day. If a single discharge is used, that made before breakfast is to be preferred. Cases which exhibit a *trace* only of albumin in the morning urine will sometimes show a larger quantity in a sample passed after active exercise.

For accurate results, it is necessary to take a portion of the *total quantity* passed in the twenty-four hours. To obtain this, the patient is instructed to throw away the urine voided at an hour noted, say 9 A. M. Every evacuation of the bladder after this hour must be made into one vessel, such as a large "specie jar," which should be well cleaned daily and kept in a cool place.

At the same hour of the following day the urine is passed into this collecting jar, and the amount measured

and noted. The mean daily discharge is 1250 c.c. or 40–50 fluidounces, or 2 1/2 pints. About 1/2 of a pint of this should be put into a clean glass bottle, with a fresh stopper, and examined within twelve hours at furthest. As a rule, it is best to examine within six hours after emission. The separation of sediment can be obtained in a few minutes by rotating the sample of urine in a *centrifuge*. All suspended matter is thrown to the bottom in three minutes. Ordinarily , it is allowed to subside spontaneously by standing several hours. (See p. 12.

Urine kept in a warm place soon passes into putrefaction, and then is not suited for analysis. Putrefactive changes can be retarded by adding to 1/2 of a pint of urine 10 grs. of thymol, 15 grains of chloral or shaking with it chloroform 5 drops to the fluidounce. Without putrefaction some organized deposits—as blood, tube-casts, and renal epithelium—are altered if not broken up, by urines of low density. If it is desired to preserve a specimen of these organized deposits, the clear urine should be decanted, leaving the sediment, which can be washed free of stale urine by pouring upon it a filtered strong solution of potassium acetate with a small quantity of salicylic acid dissolved in it. After standing long enough to allow the sediment to fall, the clear fluid should again be decanted and a fresh portion of the salicylated acetate of potassium solution be poured on. Instead of washing with salicylated acetate of potash, it is sometimes preferable to use in the same way a solution of chloral, 15 grains to the ounce of water. The specimen in chloral can be mounted on a microscope slide and will keep indefinitely. Chloral and chloroform are both reducing

agents for Fehling's solution and should not be used if the urine is to be tested for glucose.

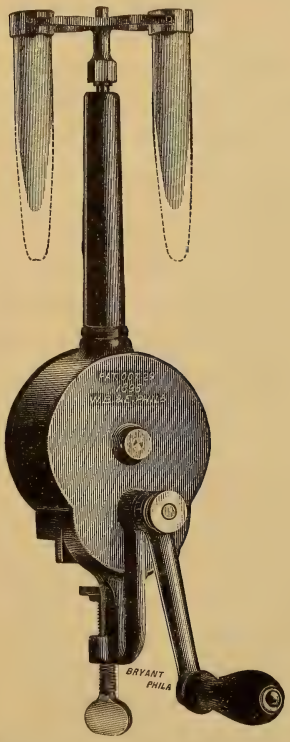

Fig. 1.—Centrifuge for the Sedimentation of Urine. One Fourth Natural Size.

Fig. 2.—Percentage Tube.

Boric acid in the proportion of five grains to four fluid-ounces of urine is a very practical preservative.

Changes in Normal Urine.—If no preservative has been added, the urine will undergo the following changes:

(1) Starting clear when voided, slightly acid, no deposit.

(2) Grows cloudy from aggregation of mucous cells and debris, still slightly acid.

(3) The slight cloud settles to form the nubecula sediment composed of mucous and epithelial cells and some reddish crystals of uric acid. Above the sediment the urine is clear and still acid.

(4) The reaction less acid, the urine cloudy from precipitated phosphates.

(5) Very turbid from phosphates and bacteria, alkaline, ammoniacal in odor. Copious sediment of triple and amorphous phosphates, bacteria, ammonium urate, and epithelial debris.

1. *Experiment.*—Having noted the characters of a sample of fresh urine, let it stand aside in a warm place for a few days, and then mark the change in odor, specific gravity, reaction, and sediment. A healthy urine may first become more acid, depositing urates and uric acid, but all samples ultimately become ammoniacal, putrid, and throw down a sediment of phosphates.

COLOR.

The normal pale amber color is due to *urochrome hematoporphyrin* and *urobilin*. The reddish color seen in many disturbances of health is imparted by *uroerythrin*. Both of these are closely related to and are derivatives of the hæmatin of the blood.

In recording color it is well to take as a standard Vogel's scale, found in large treatises. The urine is *pale yellow* after free potations, after hysteric or epileptic

attacks and in diabetes; *orange-red* by the presence of santonin when alkaline; *reddish* with scant potations, after heavy meals, much exercise, or copious sweating, after hemorrhage into any part of the urinary tract, during fever, after the administration of logwood and sulphonal; *brownish* in melanosis and after the administration of rhubarb, senna, gallic acid, and tannic acid. When tar ointment or pitch plasters are applied, and when tar and carbolic acid are given internally, compounds of sulphocarbolic acid appear in the urine. These oxidize in the air, and as the urine turns alkaline change to greenish-black pigments. The presence of biliary coloring matter imparts an intense yellow or brown or *olive-green* hue; it is characteristic of jaundice. Blood gives a *scarlet* or a smoky-brown tint.

Alkaptonuria.—A peculiar dark brown color has been observed and attributed to the presence of *alkapton*, which in the air oxidizes to a dark substance. In recent years cases have been reported which appear to be congenital. Baumann calls the color material *homogentisic* acid. It is produced by a derangement of the metabolism of proteids.

GMELIN'S TEST FOR BILE PIGMENTS.

2. Put some *yellow nitric acid—i. e.*, containing lower oxids of nitrogen—into a test-tube and overlay it with biliary urine. At the line of junction there will rise, in the following order, bands of green, blue, violet, red and yellow. The green and red will be the most persistent. This reaction is characteristic of freshly voided urines

from jaundiced subjects. It is never found in normal urine and can not be obtained in perfection from the ox-gall of the butchers. It is caused by bilirubin.

The same play of colors can be seen if the test is performed by bringing together on a white plate or piece of white filter paper the urine and the nitric acid. If the amount of bile color is very small, yielding a doubtful result, it can be concentrated by shaking a

FIG. 3.—Urinometer.

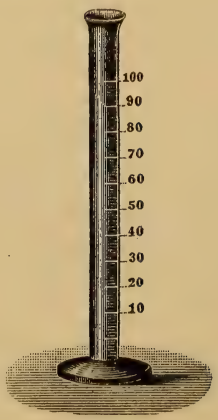

FIG. 4.—Graduated Cylinder for Measuring the Amount of Urine.

quantity of urine with chloroform and then separating the chloroform by decanting the urine. If the chloroform is now overlaid with nitric acid, the colors will appear at the line of contact.

Import.—When the bile is not freely discharged into the intestines, the coloring matter is absorbed by the lymphatics, stains the various tissues of the body, causing jaundice, and is eliminated by the urine.

3. *Oliver's Test for the Biliary Acids.*—The reagent is prepared by dissolving 30 grains of pulverized meat peptone, 4 grains of salicylic acid, and 30 minims of strong acetic acid, in sufficient water to make 8 fluid-ounces. Preserved by the salicylic acid the peptone shows no signs of decomposition on keeping. To use the test, mix 1 fluidram of the reagent with 20 minims of urine, previously diluted to a standard specific gravity of 1008. A faint haze is produced, after a minute, by the trace of biliary salts in normal urine, but if these are in excess, a milky cloud promptly appears, which will be more or less distinct according to the proportion of bile salts present.

Pettenkofer's test is not practicable for urinary analysis.

Import.—The biliary acids mean nothing different from the biliary pigment. The tests for the acids are much more difficult and do not compensate in their significance for the trouble required. It is sufficient to find the bilirubin as stated above.

4. *Urobilinuria.*—Closely related to the normal urobilin or urochrome there is a *pathological* urobilin which can be made from the normal urobilin by the action of reducing agents. Its spectrum is different from that of the normal pigment. It is identical with the *stercobilin* of the fæces and its behavior can be studied by testing an alcoholic extract of fresh human fæces.

Clinical Test.—Mix urine with an equal quantity of a

10 per cent. solution of zinc acetate in alcohol. Filter off the precipitate and the filtrate shows a beautiful fluorescence and distinct absorption spectra.

More Delicate Test.—A test-tube is filled three-fourths with urine and one drop of hydrochloric acid is added. Amyl alcohol about one fluidram (4 c.c.) is shaken slowly 8 times with the urine and allowed to separate as a top layer containing the urobilin. Pour off the clear amyl alcohol into another test-tube and add twice the amount in strong alcohol. Having prepared a 5 per cent. solution of zinc chlorid in alcohol add 1 c.c. of this to the alcohol extract of urine and then one drop of ammonium hydroxid. Zinc hydrate is precipitated and when separated by the filter, the solution containing urobilin gives a beautiful green fluorescence.

Import.—Urobilin has been detected in cases of hepatic cirrhosis, malarial anemia, carcinoma, Addison's disease, etc., when the skin was icteric in hue but no bilirubin was found in the urine.

ODOR.

It is easy to detect a departure from the familiar odor of health. Some articles of food, as asparagus, onions and some aromatic medicines, such as turpentine and copaiba, impart a change. Saccharine urine has a fruity odor. Putrid urine has the odor of ammonia modified.

SPECIFIC GRAVITY.

5. *Test.*—In taking the specific gravity, if the containing vessel has a level lip, the most accurate reading is

made by immersing the urinometer while the vessel is half full of urine, and then carefully filling it to the brim. The exact line of the registry can be taken by sighting horizontally the surface of the urine, which rises slightly about the stem of the instrument. It is best to "read off" by the *lower surface line*, and not by the point to which the liquid wets the tube, which may be a degree higher.

To make it a reliable index of the metabolic processes the specific gravity must be taken with a sample of the total urine collected for the twenty-four hours. When the observation is limited to the urine of a single act of micturition no deductions of a general character will be of any value.

For scientific accuracy, the specific gravity bottle and balance are to be preferred to the urinometer. When the amount available is small a 1 c.c. pipette may be filled and weighed. The weight of 1 c.c. = the specific gravity.

Import.—The healthy standard is between 1015 and 1025. If persistently below 1015, Bright's disease or diabetes insipidus may be suspected.

If persistently above 1030, diabetes mellitus probably exists. Further examination is necessary to make its meaning clear.

QUANTITY.

The mean daily amount is about 2 1/2 pints, or 40–50 fluidounces (1250 c.c.). It may vary in health between 1 1/2 and 4 pints.

Anuria.—Entire failure to pass urine may be due

either to *suppression* of the secretion of the kidneys, or *retention* of the urine by obstruction to the bladder or other parts of the urinary passages.

Oliguria.—Scanty urine attends all conditions associated with a lowered blood-pressure.

Hydruria.—In this condition the water is increased out of proportion to the solids.

Polyuria.—This term is applied to cases of increase in the urine as a whole, both solids and water.

Import.—Scanty urine is found in certain forms of Bright's disease, in cirrhosis of the liver, and in collapse. Hydruria is found in diabetes insipidus, and, in a minor degree, in hysteria and the middle period of atrophic nephritis.

SOLIDS OF THE URINE.

6. Roughly estimate the solid urine in parts per fluidounce by Bird's formula—*i. e.*, the last two figures of the specific gravity nearly represent the grains of solids to the fluidounce. The same figures multiplied by 2 (Trapp's factor) will give, more accurately, parts per 1000. For example, if the specific gravity is 1025, then there are 25 grains of solids to the fluidounce of urine, or 50 parts in the thousand. Haeser's factor is 2.33, which in a large number of samples is no more accurate than Trapp's.

The most exact method is to evaporate to dryness 5 c.c. in a watch crystal over sulphuric acid under the receiver of an air pump, then weigh the residue. In practice this is found too tedious for clinical use.

REACTION.

7. *Test.*—First put a drop of the sample on a piece of blue litmus paper; if the blue turns to red, then the reaction is *acid;* if the paper is unchanged, then it will be either *alkaline* or *neutral.* Now try it with red litmus paper; if it turns blue, the reaction is *alkaline;* if the paper is unchanged, the urine is *neutral.* Violet-colored "neutral litmus" paper can be used; it will turn reddish with acids and bluish with alkalies. A urine containing a certain definite proportion of the acid sodium phosphate NaH_2PO_4 and the feebly alkaline phosphate Na_2HPO_4 will give the *amphoteric reaction, i. e.,* turn blue litmus red and red litmus blue.

The normal reaction is acid, due to acid salts, such as acid sodium phosphate, NaH_2PO_4.

If alkaline, it may be so from salts of potassium and sodium, such as Na_2HPO_4 (fixed alkali), or from ammonia (volatile alkali). If due to ammonia, then by exposure the ammonia vaporizes and the red color of the paper is restored; whereas, the blue from fixed alkali remains.

While the normal reaction of the total daily discharge is acid, it can be made alkaline by the administration of alkaline medicines.

Urine is made ammoniacal by the decomposition of urea brought about by the enzym *urase* produced during the growth of the *micrococcus ureæ.*

$$\underset{\text{Urea.}}{CON_2H_4} + \underset{\text{Water.}}{2H_2O} = \underset{\text{Ammonium carbonate.}}{(HN_4)_2.CO_3.}$$

Determination of Acidity.

As the acid reaction of the urine is caused exclusively by the acid phosphate the ordinary method of acidimetry is not now considered suitable nor accurate.

8. *Freund's method* expresses the degree of acidity in terms of phosphoric anhydrid (P_2O_5) existing in the form of acid phosphates (NaH_2PO_4). The urine of the day is kept in cold storage until the total is collected. An estimate is made of the total amount of phosphoric anhydrid by operating on 50 c.c. after the method given in § 22, page 22. This is noted as (T). A second portion of 50 c.c. is taken to estimate the dimetallic phosphates (M) by precipitating with 10 c.c. of normal barium chlorid (122 grams $BaCl_2 2HO$ in 1000 c.c. of water) for every 100 mgrms. of (T). The monometallic phosphates (D) remain in solution. The mixture is diluted to 100 c.c., shaken, filtered till clear and the phosphoric anhydrid determination is made the second time on 50 c.c. of the filtrate, subtracting 3 per cent. for partial conversion, the remainder (D) is the phosphoric anhydrid present as monometallic phosphates. The total amount of phosphoric anhydrid fluctuates widely, hence it is desirable, for comparison, to know the relative proportions per cent. of urine of (T) to (D) and also the absolute degree of acidity.

Example.—If T was 0.375 grams per 100 c.c. of urine and D. was 0.325 grams. Deducting 3 per cent. of D leaves the value of D to be 0.315. Then the relative proportion of T to D would be 84 calculated as follows:

$$0.375 : 0.315 :: 100 : x = 84.$$

If the total daily quantity was 1500 c.c. then the absolute degree of acidity would be 1260 thus:

$$100 : 84 :: 1500 : x = 1260.$$

To express the result in terms of hydrochloric acid, 100 mgrms. of the monometallic phosphate correspond to 102.8 of hydrochloric acid. Should the urine be alkaline, the whole operation must be the same with the difference that decinormal hydrochloric acid is first carefully added until the cloudy sediment disappears, showing excess of acid. Then the amount thus added must be deducted from the total acidity at the end of the operation. If the results leave a negative value this may be expressed in terms of sodium hydrate.

Import.—When the alkalinity is due to ammonia, there is danger of inflammation of the bladder and a deposit of mixed phosphates occurs. If these deposits are not removed by washing out the bladder, they will in time form a calculus.

CHLORIDS.

The chlorin in the urine is in combination principally with sodium as NaCl. The mean daily discharge is 200 grs., or 12 gms., amounting to more than all the other mineral salts together. The chlorids diminish in acute fever when exudations are forming, and in croupous pneumonia may disappear entirely to reappear with convalescence.

9. *Test for Chlorids.*—First, add a few drops of nitric acid to prevent the precipitation of phosphates, then on the addition gradually of solution of silver nitrate, a

heavy white precipitate of silver chlorid forms if the chlorids are abundant, but if scanty, then a milky appearance only.

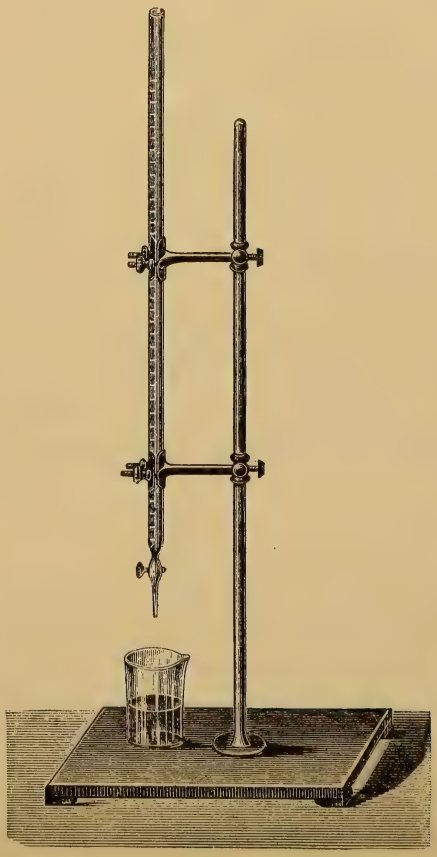

Fig. 5.—Graduated Buret.

By comparison of this precipitate with that from a sam-

ple of normal urine a rough estimate can be made of the relative amount of chlorids.

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$

10. *Volumetric Determination.*—See page 32 for rules to be observed. Dilute 10 c.c. of urine with 50 c.c. of water. Put this into a beaker or capsule, with 8 or 10 drops of neutral potassium chromate. Add gradually from a buret (Fig. 5) the standard solution of silver nitrate until the urine is permanently orange-red; then note the cubic centimeters of reagent used, subtracting 1 c.c. for excess of silver solution precipitated by uric acid, xanthin bases and pigment. The standard solution contained $AgNO_3$ 29.06 gms. per 1000 equal to 10 gms. NaCl. As 1 c.c. =0.01 NaCl in the 10 c.c. of urine used, or 0.1 per cent., by multiplying the number of cubic centimeters noted by 10, the percentage can be obtained. If percentage is multiplied by 4.55 we get the number of grains to the fluidounce of urine. Then, if 15 c.c. were used, 14 c.c. would be counted.

1. c.c. = 0.01 NaCl in 10 c.c. of urine used.
14 c.c. = 0.14 NaCl in 10 c.c. of urine used.
0.14 × 10 = 1.40 NaCl in 100 c.c. of urine used.

1.40 per cent. multiplied by 4.55=6.37 grs. to the fluidounce.

This method, which serves well enough for clinical study, can not be considered absolutely exact. To make it so, the organic compounds may be removed.

11. *Incineration Method.*—Into a small platinum or porcelain dish is placed 10 c.c. of urine and about 2 grams of pure potassium nitrate. Carefully evaporate to dryness without stirring. When dry, heat the residue over

a strong flame to a colorless fused mass. Dissolve in 30 c.c. of water and 10 drops of nitric acid.

The solution is put in a small beaker and neutralized by adding calcium carbonate until effervescence ceases. Now apply the volumetric determination section 8 with standard silver nitrate using potassium chromate as indicator, and counting every c.c. of silver nitrate without deduction. If 6.2 c.c. were used then 6.2×0.010 gram NaCl = 0.062 gm. NaCl in the 10 c.c. of urine. In 100 c.c. there would be 0.62 gm. NaCl and 0.62 per cent. multiplied by 4.55 = 2.82 grains to the fluidounce.

12. *Centrifuge Estimation.*—Filter the urine if cloudy, and fill the percentage tube to 10 c.c.; acidulate with 1 c.c. of HNO_3, and add 4 c.c. of strong $AgNO_3$; invert three times; stand aside three minutes; rotate three minutes; read every 1/10 c.c. as 1 per cent. by bulk. The normal is 10 per cent. or 1 c.c. Every 1/10 c.c. corresponds to 0.13 per cent. of NaCl by weight or 0.62 grs. to 1 fluidounce. The normal proportion of NaCl by weight is 1 per cent.

SULPHATES.

The compounds of sulphur derived from food make the *mineral* or preformed sulphates and the oxidation of proteids of the body cause the formation of *conjugate* or ethereal sulphates. They are discharged daily to the amount of about 30 grs. or 2 gms., mostly in the *mineral sulphates* with simple alkaline bases ($K_2SO_4 + Na_2SO_4$), but partly in organic compounds, called the *conjugate sulphates* of potassium, such as indican (indoxyl potas-

sium sulphate), phenol potassium sulphate, skatoxyl potassium sulphate and allied compounds.

The mineral sulphates, which make about nine-tenths, can be detected by the following test:

13. *Test for Preformed Sulphates.*—Into the test-tube containing the urine put a few drops of acetic acid to prevent the formation of barium phosphate. On adding solution of barium chlorid, a white precipitate falls, varying in density from a cloudiness to a creamy consistency. Compared with the results obtained from equal amounts of healthy urine acidified one can judge as to whether the sulphates are greater or less than normal.

14. *Test for Conjugate Sulphates.*—Having prepared the alkaline barium chlorid mixture by mixing 2 volumes of saturated solution of barium hydrate and 1 volume of saturated solution of barium chlorid, 25 c.c. are added to 25 c.c. of urine and after a few minutes filtered. This precipitates both phosphates and preformed sulphates. The filtrate is acidified with 5 c.c. of hydrochloric acid and boiled for some time. It soon colors red and gradually grows turbid by precipitation of the barium with the sulphuric acid liberated from the conjugate sulphates.

15. *Determination of Total Sulphuric Acid.*—It is sometimes important in the study of intestinal diseases to know not only the total sulphuric acid but also the amount of conjugate sulphate. For the first take 50 c.c. of clear filtered urine, acidify with 5 c.c. of hydrochloric acid and heat to boiling. Of boiling solution of barium chlorid saturated 10 c.c. are now added in a large beaker. The mixture is kept hot on a water bath for six hours and stood aside over night so as to permit the precipitate to

take a form that will not pass through the filter. The dried filter and its precipitate are placed in a weighed platinum or porcelain crucible and incinerated until white. When cool the crucible is put in a desiccator and then weighed. The gain in weight gives the weight of barium sulphate in 50 c.c. of urine.

16. *Determination of Conjugate Sulphates.*—Mix and stir 100 c.c. of urine with an equal quantity of alkaline solution of barium chlorid (see § 14). As soon as the abundant precipitate falls the clear fluid is poured through a dry filter into a dry graduate to the 100 c.c. mark. In the filtrate remain the conjugate sulphates which do not react with the barium salts also present, until the sulphuric acid is set free by boiling with hydrochloric acid. To the 100 c.c. of filtrate add 5 c.c. of hydrochloric acid and boil for some time. The mixture turns dark red and turbid. It is then kept on a water bath for six hours until the barium sulphate has settled. It is best to let it stand over night. The precipitate is separated on a filter, washed, dried, ignited and weighed as described above, § 15.

The weight of barium sulphate indicates the conjugate sulphates in 50 c.c. of urine and deducted from the total sulphates gives the preformed sulphates.

To ascertain the amount of sulphuric acid H_2SO_4 in the 50 c.c. multiply the weight of barium sulphate by 0.41201. To calculate SO_3 multiply the barium sulphate with 0.34301.

The ratio of conjugate sulphates to the total in normal urine is about as 1:10.

17. *Centrifuge Estimation of Mineral Sulphates.*—

The percentage tube is filled to the 10 c.c. mark with urine. Having acidulated with 2 drops of HCl, a solution of $BaCl_2$ is added to the 15 c.c. mark. Rotate three minutes, and read off every 1/10 c.c. of white precipitate as 1 per cent. of sulphates by bulk. The normal percentage is 0.8. Every 1/10 c.c. of white precipitate corresponds to 0.25 per cent. by weight of SO_3 or 1.1 grs. to the fluidounce.

18. *Indican* or potassium indoxyl sulphate. As indican is a characteristic conjugate sulphate, it will stand for the whole group; we can apply Jaffe's test given below and estimate approximately the increase above the normal by comparing the depth of blue color with that caused by a normal sample. When indican is treated with hydrochloric acid, indoxyl is liberated from the potassium sulphate. An oxidizer such as chlorine solution converts indoxyl to indigo blue. Chloroform will dissolve this and separate it from urine.

Jaffe's Test.—Indican is shown by adding to 10 c.c. of normal urine an equal volume of hydrochloric acid and 1 or 2 drops of liquor sodæ chlorinatæ or 3 drops of Aq. Hydrogenii dioxidi or a small pinch of sodium perborate. On standing several hours the color changes to bluish, from the formation of indigo-blue. By shaking with 1 c.c. of chloroform, the indigo dissolves in it and separates as a blue bottom layer. Estimate by depth of color and state as "scanty," "normal" or "excessive."

Import.—As yet no such correspondence has been detected between the total amount of mineral sulphates and any pathologic process as to make an estimate of value in diagnosis. The *ethereal sulphates* are deemed to

have clinical significance; as products of putrefactive change in the proteids of food, they are increased by constipation or other condition leading to intestinal decomposition. When the intestines are empty the urine ceases to contain indican or other conjugate sulphate. Before operating for carcinoma of the intestines, this desirable emptiness may be determined by the tests given above.

PHOSPHATES.

The phosphoric acid of urine is united partly with the earthy bases, as acid calcium and magnesium phosphates ($MgHPO_4 + Ca_3(PO_4)_2$), and partly with alkaline bases, as acid sodium phosphate and a trace of acid potassium phosphate ($NaH_2PO_4 + KH_2PO_4$). The average daily output is 60 grs. or 4 gms., of which two-thirds are joined to alkaline bases and one-third to earthy. As the latter are insoluble in alkaline fluids, they are deposited whenever the urine is made alkaline by an internal course of alkaline remedies, or by putrefaction, changing its urea into ammonium carbonate.

$$\underset{\text{Urea.}}{CON_2H_4} + 2H_2O = \underset{\text{Ammonium carbonate.}}{(NH_4)_2CO_3}.$$

$$\underset{\text{Magnesium phosphate.}}{2(MgHPO_4)} + (NH_4)_2CO_3 = H_2CO_3 + \underset{\text{Triple phosphate.}}{2(MgNH_4PO_4)}.$$

19. *Test.*—To the urine in a test-tube add a few drops of potassium hydrate and boil. The earthy phosphates are thrown out and must be separated by filtration.

20. *Test.*—To the filtrate add one-third its volume of "magnesium mixture." The precipitate formed represents the phosphoric acid once held by alkaline bases, now

in the form of ammonio-magnesium phosphate (triple phosphate).

21. *Tessier's Method.*—To get an approximate estimate of the quantity of phosphoric acid in urine, we can resort to the following easy method: Into a cylindric vessel, graduated in cubic centimeters, measure 50 c.c. of urine. To this add "magnesium mixture," a sufficient quantity—at least 15 c.c. After shaking together set aside for twenty-four hours. As triple phosphate, all the phosphoric acid will be thrown down in a compact white sediment, of which 1 c.c. in height represents 0.3 gm. per liter, or 0.03 per cent. of phosphoric acid. This is equivalent to 0.6 to 0.7 gm. of phosphates per liter, or 0.06 to 0.07 per cent.; multiplying this percentage by 4.55 will give the amount in grains to the fluidounce.

22. *Centrifuge Estimation of Phosphates.*—In the percentage tube mix 10 c.c. of urine and 5 c.c. of "magnesium mixture." Invert several times and after rotation for three minutes read off every $\frac{1}{10}$ c.c. of sediment as 1 per cent. by bulk of phosphates. The normal amount of precipitate is 8 per cent. by bulk.

Each $\frac{1}{10}$ c.c. of sediment is equal to 0.0225 per cent. by weight of P_2O_5.

22a. *Volumetric Estimation of Phosphoric Anhydrid.*—Having measured 50 c.c. of urine, pour it into a capsule resting on wire gauze or a sand-bath. Add to this 5 c.c. of a saturated solution of sodium acetate, which has an excess of acetic acid (5 grams of sodium acetate dissolved in 45 c.c. of water and enough acetic acid to make 50 c.c.). Heat to simmering, and slowly add from a buret the standard solution of uranium acetate. A precipitate forms, of

uranium phosphate, insoluble in the acetic acid. With a glass rod stir the mixture, and at intervals let a few drops fall on a solution of potassium ferrocyanid in a white plate, to see if it will form a red-brown spot, indicating that excess of uranium acetate has been added.

Note the number of cubic centimeters of standard solution used up to that point.

FIG. 6.—Deposit in Ammoniacal Urine (alkaline fermentation). *a*. Acid ammonium urate. *b*. Ammonio-magnesium phosphate. *c*. Bacterium ureæ

The standard solution of uranium acetate contained 31.1 gms. to 1000 c.c. equal to 5 gms. phosphoric acid, P_2O_5. As 1000 c.c. = 5 gms. P_2O_5, then 1 c.c. of the test will represent 0.005 gm. P_2O_5 in the 50 c.c. of urine taken. To get per cent., multiply the number of cubic centimeters used by 0.01, which is the equivalent of 0.005×2. The per cent. multiplied by 4.55 will give grains to the fluidounce.

Example.—If 8 c.c. of test solution were used then $8 \times 0.005 = 0.040$ gm. phosphoric anhydrid in 50 c.c. of urine. Multiplying 0.040 by 2 gives in the 100 of urine, 0.08, and 0.08 per cent. multiplied by 4.55 gives 0.364 grains to the fluidounce.

Import.—The quantity of phosphates in the urine depends mainly upon the amount absorbed from the food, which varies greatly. A knowledge of the relative amount of phosphates in a sample of urine is of no service in diagnosis or treatment, unless an almost impossible determination be made of the amount absorbed from food. The clinical significance of the phosphates depends not on the amount but on their state. When they are persistently thrown out from solution, there is reasonable ground to fear that in time gravel or a calculus may be formed. When the urine is retained in the bladder, and when from cystitis it becomes purulent and rapidly putrescible, passing into ammoniacal fermentation, the alkaline reaction induced by the ammonia causes the precipitation of the earthy phosphates before micturition.

Phosphatic Deposits.—The amorphous phosphate deposit occurs whenever the urine becomes alkaline, is grayish-white, and composed of the phosphates of calcium and magnesium. Under the microscope it appears as granules, without color or tendency to aggregate in masses of any particular shape.

To obtain the sediment, the urine may be set aside in a cool place for six to twelve hours. If the weather is warm chloroform 5 drops to the ounce will preserve the specimen until the deposit falls. It is removed by means of a pipette carried down with the finger on the outer opening

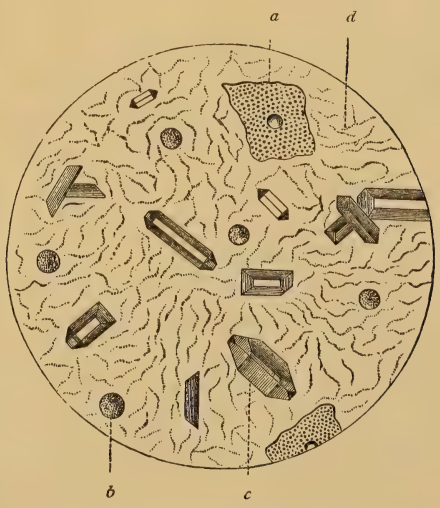

FIG. 7.—Deposit from a Case of Inflamed Bladder (ammoniacal fermentation). *a*. Detached epithelium. *b*. Pus corpuscles. *c*. Triple phosphate. *d*. Microorganisms.

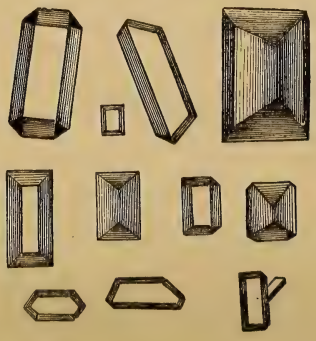

FIG. 8.—The More Usual Forms of Triple Phosphate. ×300.

until the suspected object is reached when the pressure of the finger is relaxed and the object enters the pipette. A drop or two thus obtained is spread upon the microscope slide and examined with a 1/2 or 2/3 objective without covering with a slit. When a general survey reveals particular objects of doubtful structure, a cover slip is put on and a higher objective used for the details.

The granules of amorphous *urates* usually are pinkish, arranged in delicate moss-like forms, and disappear on the addition of potassium hydrate.

Under this reagent the phosphates are unchanged, but they clear up when a drop of acetic acid is introduced under the cover-glass.

Triple or Ammonio-magnesic Phosphate.—This is the combination in which the phosphoric acid falls after putrefactive changes. To the naked eye it is a whitish sediment in a turbid fluid. Sometimes the crystals may be detected as brilliant white points on the sides of the glass, or as a shining film on the surface of the urine.

Under the microscope most of these crystals are prismatic and have some resemblance to a "coffin lid." They are colorless, bright, relatively large, and easily distinguished from the spiny spherules of ammonium urate which often accompany them.

Import.—Their presence denotes ammoniacal decomposition of the urine. If this condition exists at the time of micturition, it is evidence of cystitis or other disease of the genito-urinary tract. If persistent, there is liability to the formation of phosphatic calculus.

URIC ACID.

$C_5H_4N_4O_3$.

Uric acid, like urea, is nitrogenous. The quantity eliminated daily is 0.7 gm. or 10 grs. The normal proportion of uric acid to urea is as 1 to 45.

In healthy urine it exists only in combination as potassium and sodium urates. Acid and dense urine, on stand-

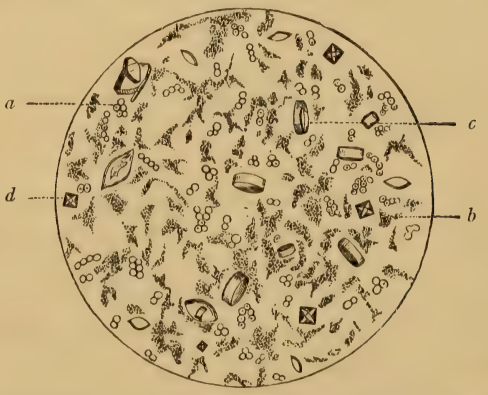

FIG. 9.—Deposit in "Acid Fermentation" of Urine. *a*. Fungus. *b*. Amorphous sodium urate. *c*. Uric acid. *d*. Calcium oxalate.

ing, will undergo change, becoming more acid and throwing down a deposit of uric acid, calcium oxalate, and amorphous sodium urate.

Free uric acid may exist in the urine at the time it is voided, but in any but the smallest amount must be considered as pathologic. It is practically insoluble, requiring 18,000 parts of water to dissolve it. It is freely soluble in the alkalies and solutions of the alkaline carbonates.

23. *Separation of Uric Acid.*—To 200 c.c. of urine add 10 c.c. of hydrochloric acid; let it stand for twenty-four hours, when the uric acid will be settled as fine crystals, looking like grains of Cayenne pepper.

24. *Estimation.*—Having obtained the crystals as above, decant the urine and stir the sediment with 30 c.c. of water, using for this purpose a glass rod with a piece or rubber tubing at the end. Throw the suspended crystals on a weighed filter, wash with water 2 c.c. at a time, keeping count of the quantity until the water ceases to show white with silver nitrate, dry over a water-bath, and weigh.

For every 10 c.c. of water used in washing the precipitate on the filter allow 0.0004 gram of uric acid lost to be added to the weight of the precipitate.

This method, though simple, is approximative only.

25. *The centrifuge* method of estimating is applied after the phosphates are separated by placing 10 c.c. of urine in the percentage tube with about 1 gm. of sodium carbonate and 1 to 2 c.c. of ammonium hydrate. The precipitated phosphates are thrown down by rotation in the centrifuge for about 5 minutes, and the clear urine poured off into another tube. To this are added 2 c.c. of ammonium hydrate and 2 c.c. of a 5 per cent. solution of silver nitrate (to which ammonium hydrate has been added until the first precipitate clears up). The mixture is inverted several times, placed in the centrifuge and the translucent precipitate of silver urate is separated by rotation. Decanting the supernatant liquid, the precipitate of silver urate is washed free of chlorid by mixing it with at least 5 c.c. of ammonium hydrate, and the mix-

ture is then rotated in the centrifuge several periods of three minutes each, until the precipitate is reduced to its least bulk. For 1/10 c.c. of this precipitate, the uric acid is read off as 0.001176 gm. in 10 c.c. of urine. To get percentage, this product is again multiplied by 10. Thus, if the reading was 0.5 c.c. then $5 \times 0.00176 =$ 0.00588 gm. in 10 c.c. or 0.0588 per cent.

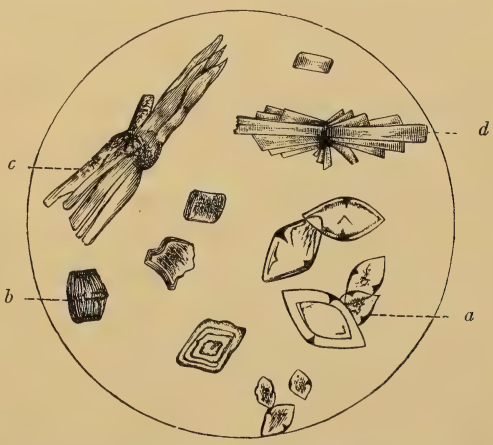

FIG. 10.—Uric Acid. *a*. Rhombic tables (whetstone form). *b*. Barrel form. *c*. Sheaves. *d*. Rosettes of whetstone crystals.

26. *Murexid Test.*—Put any substance supposed to be uric acid or urates into a watch crystal or a porcelain dish. Dissolve in strong, cold nitric acid, and at gentle heat evaporate to dryness. Touch the yellow or red residue with a drop of ammonia water, or simply expose it to the vapor of ammonia. A bright violet blue or purple red (murexid) indicates uric acid.

27. *Schiff's Test.*—Dissolve a few crystals of uric acid

in a test-tube containing a small amount of sodium carbonate solution. Having moistened white filter paper with one drop of silver nitrate, touch it with the uric acid solution and a black spot of metallic silver forms.

Deposit of Uric Acid.—While a faint trace of free uric acid may be found in healthy urine, if present in noticeable amount, it is a pathologic change. Once deposited, it remains undissolved in acid urine. It is usually colored by urinary pigment and easily made out by the microscope.

To the naked eye it appears in minute reddish specks. Under the *microscope* these specks are found to be modifications of rhombic crystals. The simpler lozenge forms have a resemblance to a *whetstone*. Some are like a drum, others are like rosettes, others again, sheaf-like bundles.

Import.—Besides its connection with the pathology of gout, uric acid is important as a deposit.

If it is deposited soon after micturition, it is an indication of a tendency to form gravel.

The Purin or Xanthin Bases.—Uric acid is a specific product of decomposition in a class of albumins known as nucleins. The mother substance is nucleic acid combined with basic groups called conjointly the *xanthin* or *alloxur* bases. These are derived from a hypothetical body containing carbon hydrogen and nitrogen $C_5H_4N_4$ termed *purin* and are closely related to uric acid, caffein and theobromin. Uric acid is tri-oxy-purin. The purin bases found in urine are xanthin, hypoxanthin, heteroxanthin paraxanthin, guanin and adenin. Together with uric acid they are called purin *bodies*. These bases in the urine

constitute only 10 per cent. as much as the uric acid. The amount in twenty-four hours varies between 0.0286 and 0.0561 gram. There is no simple method of determination that can be classed as reliable. Their relationship to pathological processes is not known. Xanthin in very rare cases is a sediment and eventually forms a calculus. The sediment is identified by the following test. A small quantity on a white plate is treated with a few drops of nitric acid and evaporated to dryness. Xanthin turns yellow and when heated with a few drops of sodium hydroxid changes to red.

URATES.

It has been said above that uric acid in health exists as neutral urates in combination with sodium principally, but with potassium partly. These urates, in ordinary amount, are soluble in normal urine at common temperatures.

If healthy urine is kept in a cold place, it loses its solvent powers, and the mixed urates are precipitated as a loose pinkish powder at the bottom of the glass and as a film on the surface.

The same thing happens at higher temperatures if the amount of urates is in excess of health and the reaction highly acid. This deposit, often referred to as sodium urate, the brick-dust or lateritious sediment, can be distinguished by its prompt solution on heating the urine. It dissolves readily in potassium hydrate.

28. *Test.*—Having obtained a specimen of urine that is turbid, fill with it a test-tube until it is half full and apply heat. It will clear up if the turbidity is due to urates.

29. *Test.*—To another portion of the same add potassium hydrate. The sediment of urates will dissolve.

Microscopically (Fig. 9, *b*), the brick-dust deposit is composed of granules usually disposed in moss-like groups. Sometimes these granules have spiny radiations.

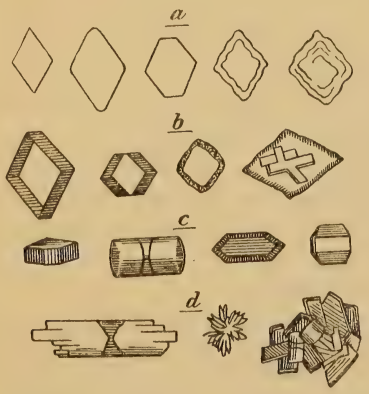

FIG. 11.—Uric acid. *a*. Rhomboidal, truncated, hexahedral, and laminated crystals. *b*. Rhombic prism, horizontally truncated angles of the rhombic prism, imperfect rhombic prisms. *c*. Prism with a hexahedral basic surface, barrel-shapped figure, prism with a hexahedral basal surface. *d*. Cylindric figure, prism with a superimposed group of crystals.

The ammonium urate (Fig. 6, *a*) occurs in opaque, brownish spherules, with or without spines, and in company with crystals of triple phosphate.

Import.—This deposit is not so important as it is commonly considered, occurring often from such physiologic conditions as unusual exercise or sweating. Pathologically, it is associated with fever and chronic hectic diseases.

HIPPURIC ACID,

$C_9H_9NO_3$,

combined with alkaline bases, is a normal constituent. The average quantity eliminated daily is 15 grs. (1 gm.). This amount is increased by excess of vegetables in the

diet or by large doses of benzoic acid. If the urine is at the same time diminished in volume, the hippuric acid may be deposited spontaneously. This is, however, an extremely rare occurrence, owing to the solubility of the acid and its salts.

It occurs in rhombic prisms (Fig. 12), resembling somewhat the coffin-lid crystals of triple phosphate. It

FIG. 12.—Hippuric Acid.

is to be recognized by the fact that it is precipitated from acid urine only, and does not dissolve when a drop of acetic acid is allowed to flow under the cover-glass. The phosphates, on the other hand, are deposited in alkaline urine and clear up in the presence of acetic acid.

CREATININ,

$C_4H_7N_3O$,

is a constituent of normal urine to the amount of about 15 grs. (1 gm.) daily. It is derived from the creatin of

muscle, is a strong base, alkaline in reaction, and, owing to its free solubility in water, is very rarely spontaneously deposited from the urine. It forms insoluble compounds with mercuric chlorid, with silver nitrate, and with zinc chlorid. The latter compound has a characteristic crystalline form, by means of which the presence of creatinin may be determined. Under the microscope

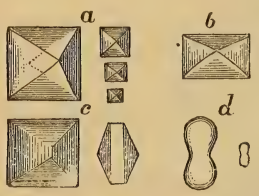

FIG. 12.—Oxalate of Lime. *a*. Octahedra. *b*. Basal plane of an octahedron forming a rectangle. *c*. Compound forms. *d*. Dumb-bells.

FIG. 14.—Perfect Dumb-bell Crystals of Oxalate of Lime.

it is seen as needles arranged in rosettes or balls. It has the power of reducing both cupric oxid and picric acid, and affects, to a slight degree, the accuracy of estimates of sugar made by reduction tests. With picric acid it forms potassium creatinin picrate, a double compound insoluble in cold water which is sometimes mistaken for albumin when picric acid is used as a reagent.

CALCIUM OXALATE.

Oxalic acid is found in extremely small amount in normal urine combined with calcium as calcium oxalate, CaC_2O_4. The normal proportion can be held in solution by acid sodium phosphate. As it takes 500,000 parts of

water to dissolve it, the slightest increase usually causes it to appear as a deposit.

30. To precipitate the dissolved portion it sometimes suffices to neutralize the urine very carefully with ammonia or simply let it stand exposed to the air. Perhaps the most delicate test for it is to treat the urine with one-third its volume of strong alcohol. In twenty-four hours the sediment is rotated in a centrifuge and examined with the microscope.

To the naked eye it appears as a scanty, grayish, mucus-like cloud in the middle or at the bottom of the glass.

Microscopically, it is recognized by one of two forms: the octahedra, usually appearing like bright, square "envelopes," and the dumb-bells or hour-glasses.

Import.—The deposit sometimes follows the eating of "rhubarb plant" or subacid fruits containing oxalates. Usually, it signifies imperfect oxidation of waste products or retarded metabolism.

If persistent, it may, by accretion, form a mulberry calculus.

CYSTIN,

$C_6H_{12}N_2S_2O_4$.

This substance, in an excessively small amount, may be a normal constituent of the urine. It is nearly insoluble in water, and in the very rare cases where it is present in large amount, it is in the form of a deposit.

To the naked eye the deposit is usually abundant and light, resembling urates. It is not dissolved by heat or vegetable acids, but readily by ammonia. The ammonia solution exposed on a glass slide deposits crystals, which,

under the *microscope*, are seen to be composed of hexagonal tablets. Iodoform escaping into the urine from surgical dressings has been mistaken for it.

31. *Test.*—When it decomposes, hydrogen sulphid is formed. If boiled with a solution of lead oxid in sodium hydrate, the black lead sulphid forms and is precipitated.

Import.—The conditions of its formation are obscure. It occurs very rarely as a calculus.

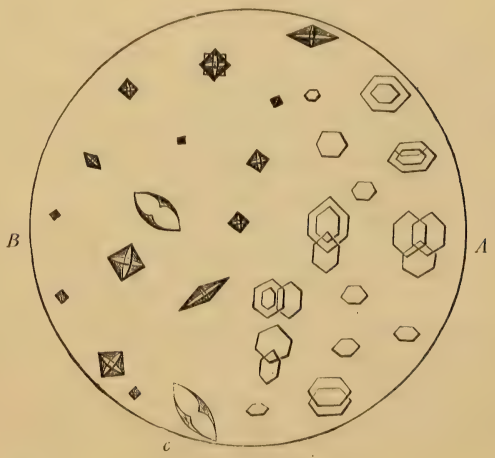

FIG. 15.—*A*. Crystals of cystin. *B*. Oxalate of lime. *c*. Hour-glass forms of *B*.

LEUCIN AND TYROSIN.

These substances are formed in the intestines by the same processes of digestion, and in acute yellow atrophy of the liver, typhoid fever, and phosphorus-poisoning appear together in the urine. In examining for these

substances, the urine should first be concentrated by evaporation on a water bath, and the cool liquid examined under the microscope.

When present, leucin will appear as a residuum left with tyrosin when the urine is allowed to evaporate on the glass slide. It occurs either as greenish-yellow globes with concentric markings or radiating spines.

FIG. 16.—*a, a.* Leucin balls. *b, b.* Tyrosin sheaves. *c.* Double balls of ammonium urate.

If a small quantity is put in a watch crystal and saturated with nitric acid, by cautious evaporation to dryness a residue will be left which turns yellow or brown on the addition of solution of sodium hydrate.

Tyrosin may be spontaneously deposited as greenish-yellow crystals composed of bundles of acicular needles arranged in various radiating forms.

Tyrosin turns red when boiled with Millon's reagent (mercuric nitrate), but yields a violet color when first carefully warmed with sulphuric acid and treated with a drop of solution of ferric chlorid.

UREA,

$CO(NH_2)_2$ Carbamid.

Urea is the chief compound of nitrogen by which that element is eliminated after having served in the body as a constituent of the proteid principles. The average daily excretion of urea is 40 gms., or 500 grs., which is about half the weight of the total solids.

It is neutral, odorless, has a bitter taste, and plays the part of either acid or basic radicle. It is easily soluble in water and alcohol, but insoluble in ether. It can be prepared by synthesis, and is isomeric with ammonium cyanate. When crystallized slowly, it forms four-sided prisms with beveled ends.

32. *Test.*—Take a specimen of urine or any fluid suspected to contain urea, concentrate it to a syrupy consistence by evaporation on a water bath, add nitric acid. If done on a slide of the microscope, the crystals viewed by a lower power are seen to be rhombic plates like Fig. 17, 3.

Estimation.—Several methods of *quantitative analysis* depend on the power of sodium hypobromite or hypochlorite to decompose urea.

$$N_2H_4CO + 3NaBrO = 3NaBr + CO_2 + 2H_2O + 2N.$$

The amount of urea involved will be indicated by the volume of nitrogen generated.

33. *Nitrogen Method.*—This method measures the nitrogen evolved by sodium hypobromite and uses apparatus which gives N in cubic centimeters, each of which represents 0.0027 gm. of urea, or apparatus graduated so as to give percentage (on the principle that 5 c.c. of a 2 per cent. urea solution will yield 37.1 c.c. of N).

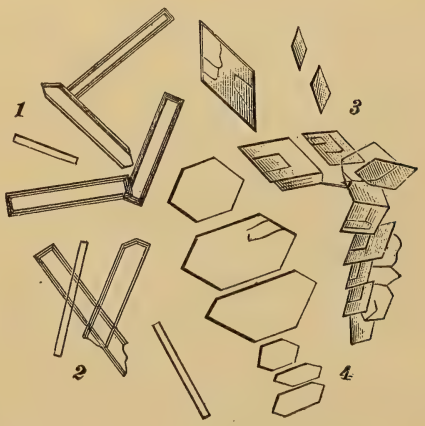

FIG. 17.—1 and 2. Urea. 3. Smaller scales ,or rhombic plates of urea nitrate. 4. Hexagonal plates.

Solutions of sodium hypobromite decompose so readily that it is best to use them freshly prepared.

Knop's Solution.—Dissolve 100 gms. of sodium hydrate in 250 c.c. of water and add 25 c.c. of bromin.

So important is it that the solution be fresh, that if any great interval is to occur between successive determinations, it is best to have the fluid in two parts, to be mixed as required. In a separate bottle keep the sodium hydrate

solution (1:2.5). To make Knop's fluid add 15 or 20 c.c. of this to 1 c.c. of bromin.

Instead of this fluid the liq. sodæ chlorinatæ, fortified by 15 c.c. of a strong solution of potassium bromid, is often employed.

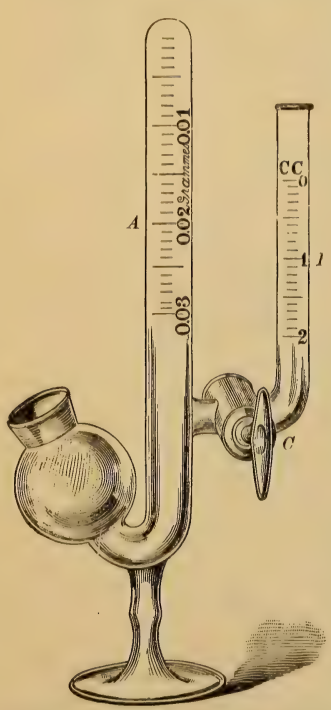

Fig. 18.—Hinds-Doremus Ureameter.

The simplest apparatus is that known as the Hinds-Doremus ureameter. (Fig. 18.) First pass the urine through the smaller attached tube so as to wet the stopcock. Having filled the larger tube with the Knop's fluid or with solution of sodium hypochlorite fortified with potassium bromid, the urine in the side tube—one cubic centimeter—is permitted to flow in drop by drop by the stop-cock to the center of the upright reagent tube. When the bubbles of nitrogen cease to rise to the upper end, the surface level at the top is read off, according to the graduation, as fractions of a gram of urea in the 1 c.c. of urine used. Thus if the surface stands at 0.018, then the 1 c.c. of urine contained 0.018 grams of urea and this multiplied by 100 gives 1.8 per cent. of urea. This multiplied by

4.55 gives grains to the fluidounce. When reading the result, the instrument must be immersed in water to the level of the column if very accurate results are sought.

34. *Apjohn's Method.*—Fill the glass cylinder (Fig. 19) with water, and immerse in it the graduated tube until the zero is at the surface. Into the flask measure 15 c.c. of hypobromite solution. A short test-tube containing 5 c.c. of urine is put in the flask by forceps, so as not to spill. Having closed the flask tightly with a rubber stopper carrying a connection with the graduated tube, tip it gradually, so as to pour out the urine into the hypobromite. Of the gas evolved, CO_2 is retained by the soda, while the N passes up the graduated tube. In ten minutes, having raised the collecting tube to the point where its contents are on a level with the water in the cylinder, read off the cubic centimeters of nitrogen. Each cubic centimeter = 0.0027 gm. of urea in 5 c.c. of urine used. If it reads 8 c.c. of N, then $8 \times 0.0027 = 0.0216$ gm. urea in 5 c.c. of urine. This multiplied by 20, to get percentage, = 0.432 per cent. Percentage multiplied by 4.55 gives grains to the fluidounce.

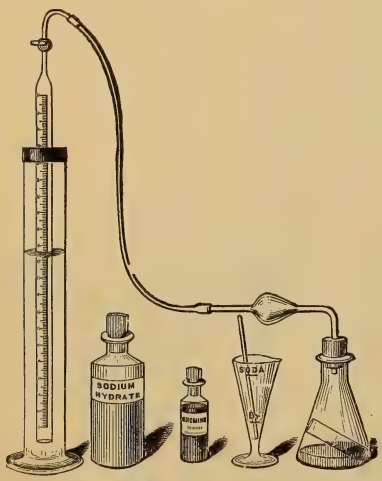

FIG. 19.—Ureameter of Charteris.

Correction for Pressure to 760 Mm. and Temperature to 0° C.—The apparatus made for clinical purposes is usually graduated so that variations in temperature, pressure and tension of aqueous vapor have so little effect on the volume of the nitrogen that they may be ignored without detriment to sound conclusions. When results are to be compared with the observations of others for perfect accuracy a correction must be made according to the following formula:

$$V' = \frac{V(b-w)}{760(1+0.00366\ T)}$$ in which

V' = volume required; v = volume observed.
b = barometer in mm.; w = tension of aqueous vapor.
T = observed temperature Centigrade.

Then to make the example given, strictly accurate when 8 c.c. of nitrogen were collected, and the barometer stood at 750 mm. the thermometer 25° C. and the tension of aqueous vapor 23.517 from table given below,

$$V' = \frac{8\times(750-23.517)}{760\times(1+0.091)} = \frac{8\times726.5}{760\times1.09} = \frac{5812}{828.40} = 7.01 \text{ c.c. of N.}$$

35. TABLE OF TENSION OF AQUEOUS VAPOR IN MM.

Temp.	Tension in Mm.	Temp.	Tension in Mm.
10° C.,	9.139	23° C.,	20.857
11° C.,	9.767	24° C.,	22.152
12° C.,	10.432	25° C.,	23.517
13° C.,	11.137	26° C.,	24.955
14° C.,	11.883	27° C.,	26.470
15° C.,	12.673	28° C.,	28.065
16° C.,	13.510	29° C.,	29.743
17° C.,	14.395	30° C.,	31.509
18° C.,	15.330	31° C.,	33.366
19° C.,	16.318	32° C.,	35.318
20° C.,	17.363	33° C.,	37.368
21° C.,	18.465	34° C.,	39.522
22° C.,	19.629	35° C.,	41.784

36. *Liebig's volumetric method* depends on the formation of an insoluble compound of urea and mercuric oxid. It is less easy than the hypobromite method, but in the absence of the special nitrogen apparatus it may be resorted to with good results. It requires several solutions: (1) The *baryta* solution, containing one volume of cold saturated solution of barium nitrate with two volumes of cold saturated solution of barium hydrate; (2) a saturated solution of sodium carbonate; (3) a *standard solution of mercuric nitrate*, which is best made by an expert chemist; it can be bought of leading druggists.

Method.—Mix 20 c.c. of baryta solution with 40 c.c. of urine, to precipitate phosphates, sulphates, and carbonates. Filter clear and put 15 c.c. of the filtrate into a beaker. Drop from a buret the standard mercuric nitrate solution, testing for excess of reagents by removing a drop at intervals and touching with it the *sodium carbonate* solution on a white plate. When a yellow spot appears, the end point has been reached.

Deduct two (for effect of chlorids) from the number of cubic centimeters used and multiply by 0.010. This gives the urea in grams contained in the ten cubic centimeters of urine operated upon. Again multiplied by ten gives percentage.

Import.—As urea is highly soluble, it is never spontaneously deposited. It varies in amount with different diseased conditions—*e. g.*, in febrile and inflammatory affections it is increased in the forming stage and diminished in that of defervescence. In diabetes mellitus and simplex it is excessive in the urine, while in acute yellow atrophy of the liver it may be absent entirely.

In acute and chronic Bright's disease there may be a decided falling off from the healthy proportion, causing a lower specific gravity. In such cases there is more or less danger of uremia. By its ready conversion into irritating ammonium carbonate it has pathologic significance in all cases of retention of urine.

SUGAR, GLUCOSE (DEXTROSE).

A minute trace of sugar can sometimes be detected in healthy urine by the use of special tests of great delicacy, but *normal urine contains no amount of sugar that can be recognized by the usual clinical tests.* The reducing power possessed by normal urine (equal to that of a 0.1 per cent. solution of glucose) is due partly to uric acid and partly to creatinin. In diabetes mellitus this power of reducing metallic salts and other compounds is enormously increased, is easily appreciated by ordinary tests, and is undoubtedly due to grape sugar, which may be present in the great amount of 50 grs. to the fluidounce, or 10 per cent., though it passes 4 per cent. in severe cases only.

The analytical reactions of glucose are based upon the following properties:

(1) In hot alkaline fluids it reduces the oxids of copper and bismuth.

(2) With brewers' yeast it ferments, yielding alcohol and carbon dioxid.

(3) It forms star-like insoluble crystals of glucosazon with phenylhydrazin.

(4) Its solutions deflect polarized light to the light.

In the application of any test but that of fermentation it is necessary to make sure that the urine is *free from albumin.* If any is present, boil the sample with a drop of acetic acid, and filter.

Reduction Tests.—The best reduction tests for glucose are such as Böttger's and Fehling's, which make use of its power to reduce metallic salts to lower oxids or the metallic state when boiled with them in alkaline solution. The most conclusive test is by fermentation and this should be used whenever the reduction tests give a doubtful result.

37. *Böttger's Test.*—(First, test for albumin; if present, separate it by acidulating the urine slightly, boiling and filtering.) Put into a test-tube equal volumes of suspected urine and potassium hydroxid, with a pinch of bismuth subnitrate. On boiling for a few minutes, if sugar is present, the white powder turns gray, brown, or black, from reduction to metallic bismuth.

Fallacies.—A slight reduction of the bismuth subnitrate may be caused by turpentine, tincture of eucalyptus, kairin and large amounts of quinin. Albumin and sulphur compounds in the urine may cause a black precipitate. To make sure, repeat the test, using litharge instead of bismuth. If this blackens, then sulphur compounds have affected the bismuth also, and some other test must be used.

38. *Nylander* performs this test with a single alkaline bismuth oxid solution, made as follows:

℞	Bismuth subnitr.,	2 gms.	
	Rochelle salt,	4 gms.	
	Caustic soda (sol. of 8 per cent.),	100 gms.	Mix.

Having made sure of the absence of albumin, to 10 c.c. of urine add 1 c.c. of test solution and boil. A brown or black color denotes sugar.

Delicacy.—This test is so delicate as to show 0.025 per cent. of sugar.

Tests by Copper Sulphate.

The reducing action of glucose on copper sulphate is subject to conditions which will be understood by performing the following experiments:

39. In a test-tube add to a solution of copper sulphate an equal amount of potassium hydroxid; a pale blue precipitate of cupric hydroxid (Cu_2HO) forms, insoluble in excess. On boiling the blue hydroxid changes to black oxid, $Cu_2HO = CuO + H_2O$.

40. Put into a test-tube a solution of copper sulphate and a few drops of glycerin (or instead of the glycerin a few grains of neutral potassium tartrate or of Rochelle salt). Now add potassium hydroxid cautiously; at first a blue precipitate falls, redissolved on the addition of more potassium hydroxid. Boiling makes no change in the clear blue fluid.

This property of holding the cupric hydroxid in solution is shared by many organic compounds, as glycerin, the tartrates, and glucose; but, when boiled with it, glucose has the peculiar property of abstracting oxygen from the cupric hydrate, forming the red or cuprous oxid Cu_2O.

41. *Trommer's Test.*—To ½ of an inch of urine in a test-tube add an inch of potassium hydroxid and a few drops of solution of copper sulphate enough to cause blue color and a slight cloudiness of pale blue hydrate. Heat

just to the first signs of boiling. If sugar is present, yellow or red cuprous oxid is precipitated. When a simple aqueous solution of glucose is used red cuprous oxid is obtained, but if the glucose is dissolved in urine the yellow modification occurs from the action of some unknown urinary constituent. Slight precipitations which occur only after prolonged boiling or after the test has been standing are not proof of glucose. Normal urine may cause them from the action of uric acid and creatinin.

Delicacy.—Trommer's test carefully performed shows glucose when present 0.0025 per cent.

42. *Glycerin Cupric Test.*—To an inch of potassium hydroxid in a test-tube add a few drops of copper sulphate and a few drops of glycerin. Boil and add suspected urine by small amounts up to less than one inch in the tube. Sugar will throw out the cuprous oxid, red or yellow.

For convenience and simplicity, the copper sulphate may be dissolved, 28 grs. in a fluidounce of a mixture of equal parts of water and pure glycerin, and a few drops used as above.

This *glycerin cupric* solution keeps for some time, and takes the place of two different fluids.

43. *Fehling's Test.*—To obviate some of the defects of Trommer's test, Fehling's solution is employed. As it does not keep well, it is best to make it in two parts, not to be mixed until the time of use.

A.	Take copper sulphate,	34.64	gms.	
	Water enough to make,	500	c.c.	Mix.
B.	Pure Rochelle salt,	173	gms.	
	Sol. sodium hydroxid (specific gravity 1.33),	100	c.c.	
	Water enough to make,	500	c.c.	Mix.

For use, mix equal volumes of A and B, and thus make Fehling's solution.

44. *Glycerin-Fehling's Solution.*—Instead of Rochelle salt, glycerin can be used and the solution made as follows in one fluid which will keep some time, though not indefinitely: Dissolve 34.64 gms. of copper sulphate in 500 c.c. of water. Add glycerin 130 gms., potassium hydroxid 130 gms. and enough water to make one liter. Each cubic centimeter will equal 0.05 gm. of glucose.

45. *Method.*—Boil in a test-tube one inch of Fehling's solution. While hot, add urine and bring to a boil again. If no red or yellow color appears, add more urine until the total volume is $1\frac{3}{4}$ inches. Again raise to boiling point and set aside. If yellow or red cuprous oxid appears, the urine is saccharine.

Precautions.—Let the volume of urine be less than that of the test solution. Heat up to the boiling point without continuing the boiling.

Fallacies.—If these precautions are not observed, high-colored, acid, dense urines may reduce the copper sulphate without the presence of sugar. The reducing substances to be guarded against are uric acid, urates, creatinin, hippuric acid, mucin, hypoxanthin, glycuronic acid, alkapton, alkaloids, arsenic and carbolic acid.

Delicacy.—Fehling's test is extremely delicate, showing 0.0008 per cent. of glucose.

46. *Volumetric Estimation.*—Measure into a capsule 10 c.c. of Fehling's solution and 40 c.c. of water. Heat over wire gauze to boiling, and then, from a buret containing a mixture of 1 volume of urine and 9 of water slowly let it run into the capsule drop by drop until the

blue color of the test solution disappears in the clear line around the surface. Note the number of cubic centimeters used, and then repeat the experiment with fresh materials to see if a less amount will not answer.

Thus 10 c.c. of standard solution is decolorized by 0.05 gm. glucose. If 8 c.c. of dilute urine were used, then of the undilute 0.8 c.c. =0.05 gm. glucose. If 1500 c.c. were voided in the day, then 0.8 : 0.05 :: 1500: x=93.75 gms. To calculate per cent., 0.8 : 0.05 : : 100 : x=6.25. Parts per cent. multiplied by 4.55 give grains to the fluidounce.

Precautions.—The urine should be added drop by drop without stirring so as to get a prompt settling of the precipitate. Larger quantities disturb the blue fluid and prevent a quick estimate. The Fehling's solution should be kept simmering until the blue color appears to be discharged, the lamp is then withdrawn a moment to let the solution clear around the periphery. The end of the reaction is reached when the blue color disappears at the surface. If the blue color persists, heat to boiling again and add more urine as before. If the solution stands it will take up oxygen and turn blue again.

Objections to this Method.—Owing to the deep color of the precipitate of cuprous oxid, the end-point is masked and difficult to recognize. Only a practiced hand is sure of an accurate result. For the student and for the physician in clinical analyses it is better to use Pavy's solution as modified by Purdy and according to the following method:

47. *Copper Quantitative Test with Purdy's Solution.*—Take copper sulphate 4.752 grams; glycerin 38 c.c.; dissolve in 200 c.c. of water by gentle heat. In another

200 c.c. of water dissolve potassium hydroxid 23.5 grams, and mix with the copper solution. When cool add strong ammonia (s.g. 0.88) 450 c.c. and water to make 1000 c.c.

Method.—In a beaker or boiling flask of 250 c.c. capacity, put 35 c.c. of above solution and 70 c.c. of water. Boil over wire gauze steadily, and add slowly the urine from the buret until the blue color fades. Now after each drop wait a few seconds to see if the end of all blueness has been reached. Continue the dropping until the blue liquid is colorless and transparent. To get this result the total urine employed must have contained 0.02 grams of glucose. If the quantity of urine used was 5 c.c. then 5 c.c. =0.02 of glucose or 1 c.c. =0.004 and 100 c.c. =0.4 per cent. If the color is discharged by less than 4 c.c. of urine it is best to dilute the urine by adding 2 parts of water to 1 of urine; the result must then be multiplied by 3.

The advantages of this method are that the end-point is definite, the solution stable and the procedure rapid and accurate. The reading of the buret must be taken promptly as the test solution on standing takes up copper and again turns blue.

48. *Fermentation Test for Quantity.*—Roberts' Differential Density Method: Mix well urine enough to float the urinometer, say 4 fluidounces (120 c.c.) with half a cake of compressed yeast; take the specific gravity and record it; set aside in a warm place (mantel-piece) for twenty-four hours. After the fermentation, take the specific gravity again and subtract the new reading from that taken before. Each degree of the remainder showing the density lost by fermentation represents one grain

of glucose to the fluidounce. Multiply by 0.219 to get percentage.

Thus: Specific gravity before fermentation, 1035
Specific gravity after fermentation, 1015.
1035 − 1015 = 20 degrees of density lost, or 20 grs. of sugar to the fluidounce.

This test is very easily performed, and conclusive as to the presence of sugar, though it is not strictly accurate as to quantity, nor trustworthy when the amount of sugar is less than 0.5 per cent. Maltose, lactose and fructose ferment with compressed yeast, but much more slowly. Any reduction of density in a few hours is certainly due to glucose.

49. *Fermentation Saccharometer.*—None of the urinary substances which give reactions of reduction resembling those of sugar are susceptible of the vinous fermentation. When sugar is present and the urine is mixed thoroughly with brewers' yeast and kept in a warm place (about 100° F.) for a day, it will ferment, giving off bubbles of carbon dioxid and forming alcohol by the following reaction:

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$
Glucose. Alcohol.

The fact of fermentation can be shown by collecting the carbon dioxid gas evolved. Fill a test-tube with the urine to which yeast has been added, and invert it over a saucer containing some of the same mixture. Set aside in a warm place, and if in one hour gas has accumulated, it proves the presence of glucose. In most cases gas will appear in less than half an hour. A convenient vessel for this test is shown in Fig. 20. It is tipped on the side, filled with the urine and yeast mixture, and kept in a warm

place. In twenty-four hours all the gas collects in the upright tube, which is graduated to read from 0.25 to 1 per cent., or from 1 to 5 c.c. It will not show sugar when present to a less amount than 0.1 per cent., and owing to the varying solubility of the gas in urines of variable reaction and density, it can not be considered accurate, though it is conclusive as to the presence of sugar. A few bubbles of gas may form as the result of disengagement from the yeast itself and should be disregarded. A control experiment made by mixing the yeast with normal urine will show how much allowance to make for the yeast bubbles.

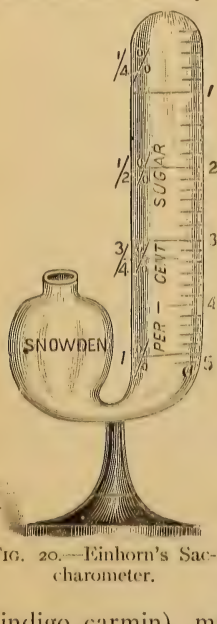

FIG. 20.—Einhorn's Saccharometer.

Fallacies.—Other carbohydrates such as lactose, maltose and levulose undergo fermentation, though more slowly than glucose. For bubbles of gas to accumulate in half an hour is certain proof that glucose is present.

Indigo Carmin Test.—A blue solution of sodium sulphindigotate (indigo carmin), made alkaline with sodium carbonate and boiled with saccharine urine, changes from blue to violet—red—straw-yellow. Cooling, or even agitation, will cause the restoration of the blue color; whereas, if the bleaching is due to strong alkalies, the color will not return. A very handy and reliable form for the test is in pellets, each of which contains the indigo carmin

and the sodium carbonate combined. The solution does not keep well.

50. *Method.*—Put in 30 minims of water one pellet of indigo carmin and sodium carbonate; heat gently to solution; let fall from a pipet *one drop* of urine and boil quietly. A change to red or yellow indicates sugar.

Precaution.—If more than one drop is added, there is a chance that a reaction will occur from healthy urine.

Methylene Blue Test.—This reagent of doubtful value is widely sold by an enterprising druggist. A clear blue solution is made by dissolving methylene blue in equal parts of water and ammonium hydrate. The suspected urine is diluted 1 part to 9 of water. Of this dilute urine 2 c.c. are added to 8 c.c. of test fluid. On boiling for two minutes the blue color disappears if glucose is present.

Fallacies.—If the fluid is shaken the blue color may return. If the urine be not diluted, it will decolorize without glucose. Normal urine 1 to 9 of water will not discharge the color, but urines rich in urates and other reducing substances may give a doubtful reaction even when diluted and free from glucose.

51. *Phenylhydrazin Test.*—Into a test-tube put nearly half an inch of dry phenylhydrazin hydrochlorate, add pulverized sodium acetate an equal volume, and then fill the tube half full with urine. The sodium acetate is dissolved, by heating up to the boiling point. Boil for *two minutes* and set aside for about 20 minutes for the yellow sediment to fall.

Remove a portion of the sediment to the slide of a microscope, and examine with a power of 200 diameters.

A quicker method is not to wait 20 minutes but put a drop of the liquid *directly* upon the slide without a cover and see the crystals form in 2 or 3 minutes.

If sugar is present, needle-shaped crystals of phenyl-glucosazon will have formed, as yellow branching sprays or sheaves or radiating stars. When the glucose is present in very small amounts, it may require hours for the fine crystals to fall in sufficient quantities for recognition. The red globules and large yellow plates often seen are not significant. Performed in the way given above this test is most reliable. It gives no reaction with normal urine, nor with urine containing pyrocatechin, maltose, lactose, glycuronic acid or pentose as seen in medical practice. Especial care must be taken in observing the microscopic forms of the radiating bundles of needles.

Delicacy.—Glucose gives characteristic crystals when present in 0.025 per cent.

52. *Polarimetric Test.*—Because of its property of turning the plane of polarized light to the right glucose is called *dextrose.* With a polariscope the estimation is made accurately and conveniently. The degree of dextro-rotation may be read upon a graduated circle and calculated as percentage of glucose. As polariscopes are expensive they do not form a part of the physician's laboratory, though in very doubtful cases resort may be had to a laboratory furnished with some one of the numerous constructions in use.

As albumin affects polarized light the urine must first be freed from it by boiling and filtering. To make an accurate adjustment of the instrument the urine must be

decolorized. A simple method is to shake with a piece of lead acetate and filter. Another method is to put $\frac{1}{2}$ c.c. of washed blood-charcoal in a test-tube, fill with urine, shake and filter. The quantity of charcoal is so small that no appreciable amount of glucose is removed.

Fallacies.—Maltose very rarely occurs in the urine but it rotates the polarized ray more than glucose. Its osazon is different from that of glucose and it does not ferment nearly so readily. Sometimes glucose is accompanied by beta-oxy-butyric acid which turns the ray to the left and hence may reduce the glucose reading or neutralize it altogether. But glucose can be identified by the other tests including fermentation. After fermentation the loss of dextro-rotation shows that glucose had been present.

Delicacy.—As instruments differ in the method of fixing the end-point, they vary in delicacy. Usually distinct dextro-rotation is determined when glucose is present 0.025 per cent.

Clinical Methods.—Glucose in small amounts can not be positively identified by any single test. If no darkening of the bismuth occurs in Böttger's or Nylander's test as applied to albumin-free urine then pathological quantities of glucose are not present. If after prolonged boiling the bismuth has turned gray or black then the fermentation test should be applied to a fresh portion. If loss of density or accumulation of carbon dioxid gas does not occur in a few hours either there is no glucose or the amount is less than 0.5 per cent. or there are antiferments in the urine due to the administration of drugs, such as salicylic acid, salol, arbutin, etc. After identi-

fication, the copper test with Purdy's solution § 47 will serve for quantitative determinations.

Import of Glycosuria.—If glucose is detected by any of the tests given above, it is pathologic. If abundant and *persistent*, and associated with excessive drinking and eating on the part of the patient, who is emaciating at the same time, it denotes typical diabetes mellitus. The urine in this disease is usually pale, of a fruity odor, and very abundant. It may amount to over two gallons or 10,000 c.c. daily; and, notwithstanding the large volume, the presence of sugar makes it dense, the specific gravity ranging from 1025 to 1045.

Temporary, slight glycosuria may follow an excessive saccharine diet, and reducing substances simulating glucose, such as glycuronic acid, may appear in the urine after the administration of chloroform, ether, chloral, amyl nitrate, turpentine, salicylic acid, benzoic acid, glycerin, camphor, sulphonal, and carbolic acid; also after poisoning from strychnin, morphin, arsenic, phosphorus, or carbon monoxid. These reducing derivatives do not, however, undergo yeasty fermentation, as glucose does, nor do they respond to the phenylhydrazin test.

It may be *incidental* to various diseases of the brain and spinal cord, and accompany phthisis, pneumonia, asthma, cirrhosis, cholera, and even be intermittent, like malarial paroxysms.

53. *Lactose.*—Milk sugar occurs occasionally in the urine of nursing women and sometimes toward the end of gestation.

Test.—It is present if a sample of urine reduces Nylander's and Fehling's solutions, but gives a negative

result to phenylhydrazin and does not yield carbon dioxid gas within several hours after the yeast ferment has been added. It is dextro-rotatory.

54. *Maltose.*—Malt sugar has been observed in the urine of some diabetic patients. It reduces Nylander's and Fehling's solutions and also ferments with yeast. With phenylhydrazin it forms a maltosazon with a different microscopic appearance from glucosazon which has also a melting point of 205° C. while maltosazon melts at 190° C. It is dextro-rotatory.

55. *Pentoses.*—Diabetics, morphin users and some healthy subjects as a metabolic anomaly occasionally excrete with their urine traces of the pentoses. The urine reduces Nylander's and Fehling's solutions but does not ferment with yeast. Pentosazons are readily distinguished by the low melting point, 160° C.

Orcin Test.—Having dissolved a few grains of orcin in 5 c.c. of strong hydrochloric acid by the aid of heat, divide into two equal parts. In one put 0.5 c.c. of the suspected urine, in the other the same quantity of normal urine. Stand both in a beaker of boiling water. Pentoses cause a green color beginning at the top and spreading downward.

ACETONE AND DIACETIC ACID.

Sometimes in advanced diabetes, in fever, or even in health, when the diet is rich in meat, the urine has an ethereal odor due to acetone. In the course of diabetes a sudden decline in the amount of sugar and urea, with a corresponding fall in the specific gravity but without

other improvement in the symptoms, may be indicative of an impending coma—a complication of the most dangerous character. At this time there will appear in the urine acetone with or without ethyldiacetic acid and oxybutyric acid. The sample will yield a wine-red color with *liquor ferri chloridi*—probably due to a reaction with diacetic acid.

56. The test is performed on 3 c.c. of urine by adding one or two drops of strong ferric chlorid, thus precipitating a yellowish phosphate of iron, which can be separated by filtration.

On adding more ferric chlorid if a marked port-wine color appears, we may safely infer the presence of the significant substance, the patient not taking antipyrin, salicylic acid, or other coal-tar product. When the change of color is slight, the same test should be performed with a sample of normal urine and the two test-tubes compared. The test is controlled by another fresh portion of suspected urine; it is first boiled, to destroy the diacetic acid, and treated as above; the disappearance of the diacetic acid is shown if there is no red color developed. Should this second sample yield no reaction, a third portion must be treated with dilute sulphuric acid, shaken with ether, and the ethereal extract tested as above with ferric chlorid. A red color, which disappears on standing a day or two, indicates diacetic acid liberated from combination.

57. *Legal's Test.*—To five cubic centimeters of urine in a test-tube add several drops of a freshly made *strong* solution of sodium nitroprussid and a few drops of sodium hydrate. A red color appears in all urines and

soon changes to yellow. Holding the tube slanting, a few drops of strong glacial acetic acid are allowed to trickle down the side so as to form a separate layer. A change of color from the yellow to crimson or purplish red at the line of junction indicates acetone. On standing a blue-green color of Prussian blue is developed, owing to the creatinin decomposing the nitroprussid.

58. *Oxybutyric Acid* can not be detected easily by chemical tests without taking a week to separate it from the other urinary constituents. As it rotates the polarized ray to the left, a sample of urine which has been freed from sugar by the yeast fermentation, and then turns the ray to the left would indicate this acid.

ALBUMIN.

In addition to the serum albumin and serum globulin, which are congeners and have the same serious significance, other protein principles of different import may occur in the urine, such as mucin (nucleo-albumin), peptones, albumoses. The albumin of the blood serum occurring in the urine is abnormal, and is nearly always evidence of grave disease. It is detected by the use of heat and of various reagents which throw it out of solution as a white precipitate. In case of turbid urine it is necessary to filter the urine clear before testing. If the urine is not clear, make it so by putting a cone of filter paper in the test-tube and dropping the urine through. If the urine is turbid from urates, filtration may be dispensed with if the boiling test is used; gentle heat first causes the disappearance of the urates, and as the temperature ap-

proaches the boiling point, the albumin coagulates. *The most highly valued tests are nitric acid and boiling heat, the most trust-worthy single test being the boiling of an acid specimen.*

59. *Nitric Acid Test, Heller's Method.*—Put one inch of urine in a test-tube and gradually run 1/4 of an inch of nitric acid down the side while the tube is inclined, so as to prevent mixing. The acid is heavier and forms a bottom layer. Wait ten minutes for the appearance of a white belt or zone *at the line of contact* of urine and acid. This is a coagulum of albumin. Although this is not the most delicate test, it is highly convenient, and albuminuria is rarely serious unless decided enough to be shown by this "contact" method.

A more definite result can be had if the nitric acid is introduced at the bottom of the urine by means of a charged pipet. A rose-red zone is usually seen from the reaction with a normal chromogen, *urorosein.*

In dense urines a haziness may appear in the upper part of the urine, 1/2 of an inch above the contact line, due to acid urates. Mucin (nucleo-albumin), if very abundant, may make a haze about 1/3 of an inch above the line of contact. Heat will clear up the urate cloud, whereas albumin will remain undissolved. In case of doubt try another sample diluted one-half.

60. *Roberts' Nitric Magnesian Reagent.*—This reagent has been devised in order to obviate objections to handling nitric acid, which, if undiluted, is a corrosive liquid not conveniently portable. It can be kept in a cork-stoppered bottle and does not stain the hands. To 5 volumes of a filtered saturated solution of magnesic

sulphate, prepared by dissolving 10 parts of the salt in 13 parts of distilled water, add 1 volume of strong nitric acid. A couple of drams of bright filtered urine is allowed to float on an equal quantity of this solution in a test-tube; care being taken that the contact line is sharply defined. In a period of time varying from a few seconds to a quarter of an hour, according to the amount of albumin present, a delicate opalescent zone forms at the point of junction, and if mucin also is present, a more diffused haze *higher up* in the urine. Special attention should be given to the position of the opacity. In some concentrated urines a belt of urates will appear a little above the line of demarcation; but these dissolve on warming.

61. *Boiling Test.*—First test the reaction. If acid, proceed at once to apply heat. If the urine is alkaline, then to a tube containing three inches of urine add one drop of acetic or nitric acid. Heat the upper half to boiling. If contrasted with the lower half, it has a turbid appearance, then albumin is present. Albumin coagulates in acid fluids at 70° C. (160° F.), but remains dissolved if the urine is alkaline. Serum albumin and its associate globulin are the only proteids that coagulate at this temperature. *This is the most single trustworthy test.*

Fallacy.—Phosphates are sometimes precipitated by boiling off CO_2 from a slightly acid specimen. Hence it is necessary to add nitric acid if a coagulum appears. It is best in all doubtful cases to use both tests—nitric acid and boiling.

Precaution: Sometimes, when acetic acid is added to a urine already acid, the albumin is converted into acid albuminate, a form noncoagulable even on boiling. The

acetic acid should not be employed except in an alkaline urine and even then only a drop, just enough to neutralize the reaction or make it slightly acid. More than this might coagulate mucin.

62. *Rough Estimation.*—The urine acidulated by a drop of acetic acid is boiled for several minutes and set aside for twenty-four hours. White flakes fall in a well-defined layer, the depth of which may be expressed as a fraction of the whole fluid in the tube, thus: it is 1/10 or 1/4 moist albuminous layer.

It is a common error to count this proportion of coagulated albumin as a percentage. By percentage of albumin is meant the amount of dried albumin, which is never more than four per cent. in the urine, while the loose moist coagulum of albumin may exceed in volume one-half the fluid in the tube.

63. *Picric Acid Test.*—A saturated solution is prepared by dissolving six grains of recrystallized picric acid in a fluidounce of water with heat, and decanting the clear solution. Some of the urine is rendered perfectly clear by filtration; the tube is held inclined, and an equal volume of the picric acid solution is gently poured down the side so as to form a top layer. On gently shaking it, the yellow color will diffuse downward, and as far as it extends, the picric acid will coagulate the albumin. In the presence of albumin a more or less distinct haze is produced which on heating to the boiling point is rather intensified than otherwise.

Fallacies.—Peptones, if present, yield a similar haze, and quinin, morphin, or other alkaloids a more or less crystalline precipitate; but in both these cases the opal-

escence is completely dissipated by moderate heat. Mucin (nucleo-albumin), an unimportant constituent of most urines, is also coagulated, but much more slowly than the other proteins.

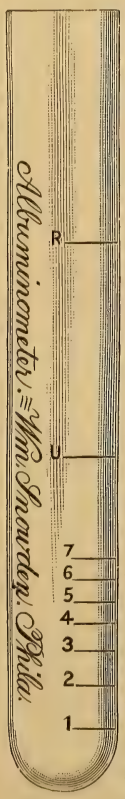

FIG. 21.

Picric acid is a sensitive test for albumin, but its clinical value is impaired by the fact that it gives a faint but perceptible reaction under conditions not serious.

64. *Gravimetric Determination.*—The only strictly accurate way of estimating the quantity of albumin is to coagulate the albumin by boiling the acidulated fluid, then after filtration, drying and weighing the precipitate. This is not suited for clinical work because of the elaborate technique, apparatus, and time required.

Esbach's Albumimeter.—It is sometimes desirable to determine the proportion present at intervals, so as to study the progress of a case. For this comparative and clinical purpose, the easy method of Esbach is to be commended. A tube graduated like that shown in Fig. 21 is to be employed. If care is taken that the urine shall be previously neutral or acid, Johnson's saturated solution of picric acid may be employed. Dr. Esbach's test solution is prepared by dissolving ten grams of picric acid and twenty grams of citric acid in 900 c.c. of boiling distilled water, and then adding, when cold, sufficient water to yield one liter. The citric acid is only employed for the purpose of maintaining the

acidity of the liquid, and is not essential except for urines decidedly alkaline. It is open to objection from its power of precipitating mucin.

The filtered acid urine is poured into the glass tube up to the mark U, and then the reagent is added until the level of the liquid stands at R. Avoid shaking, lest air bubbles form and float the clots, which it is desired to collect at the bottom. Mix the liquids thoroughly, without shaking, by slowly reversing the tube a dozen times, close with a cork, and allow it to stand upright for twenty-four hours. The height at which the coagulum then stands, read off on the scale, will indicate the number of parts per thousand, or grams of dried albumin in one liter. This divided by ten gives the percentage. If the coagulum stands above the mark 4, try again after first diluting the urine with one or two volumes of water, and then multiply the resulting figure by two or three, as the case may be. If the amount of albumin be less than 0.5 gm. per liter, it can not be accurately estimated by this method.

65. *Differential Density Method.*—This is based upon the loss of specific gravity noted when the albumin has been completely removed. A very accurate hydrometer graduated to the fourth decimal or a specific gravity bottle or a graduated 1 c.c. pipette weighed on delicate balances may be used. The ordinary urinometer is not sufficiently exact. Enough urine for the specific gravity instrument is put in an ordinary medicine bottle with sufficient acetic acid to insure coagulation of the urine. The specific gravity of the mixture is taken and recorded. The mixture is then heated to boiling and returned to the bottle which is closed by tying in a rubber stopper

that has been previously boiled in sodium hydroxid and washed. The bottle is immersed in boiling water for fifteen minutes, cooled off and the urine filtered. By covering the funnel and passing it through a cork with small nick into a bottle, loss by evaporation is prevented. Take the specific gravity of the filtrate and subtract it from that of the whole urine first recorded. The loss multiplied by 400 equals the number of grams weight of dry albumin in 100 c.c. of urine. This multiplied by 4.55 gives grains to the fluidounce.

Delicacy.—While this operation is more tedious it is said to be more exact than Esbach's albumimeter.

Example.—If the specific gravity of the original sample was 1.0234 and after removal of albumin was 1.0231 then the loss wss 0.0003. This decimal 0.0003 multiplied by 400 gives 0.12 per cent. of dried albumin by weight. Then 0.12×4.55 gives 0.546 grains to the fluidounce.

66. *Acetic Acid and Ferrocyanid Test.*—Hydroferrocyanic acid yields a precipitate immediately in the presence of much albumin, and, if traces only are present, in the course of a few minutes. To apply the test, strongly acidulate with acetic acid, and then add a few drops of recently prepared potassic ferrocyanid solution. This is one of the most delicate tests known, but is open to the objection that it precipitates albumose and mucin.

The *centrifuge* estimation by volume is performed by putting into the percentage tube 10 c.c. of urine. To this is added 1.5 c.c. of acetic acid and 3.5 c.c. of a freshly made strong solution of potassium ferrocyanid. After shaking the mixture it is rotated for three minutes. For every 1/10 c.c. of precipitate calculate 1 per cent. by

volume of albumin layer, which represents 1/60 of one per cent. of albumin by weight or 0.015 per cent.

Trichloracetic Acid.—This test is said to have more than six times the delicacy of metaphosphoric acid, and more than three and a half times that of nitric acid. It should be used in the form of a solution in water (specific gravity 1147), by superposition in the same way as Heller's nitric acid test. The white ring which forms at once is characteristic and unobscured by the colored zone of oxidized pigments as in the nitric acid test. It is too sensitive for practical purposes, sometimes showing nucleo-albumin in healthy urine. Any doubt may be resolved by boiling; albumin is the only cloud which persists.

67. *Potassiomercuric Iodid or Tanret's Test.*—The reagent is composed of mercury bichlorid, 1.35 gms., potassium iodid, 3.32 gms.; acetic acid, 20 c.c.; distilled water enough to make 1000 c.c. Used by contact, it gives an opacity with all the proteids, including mucin, and some reaction with a large proportion of normal urines. On this account, like *sodium tungstate* and *metaphosphoric acid*, it is open to fatal objections as a clinical test.

Import of Albuminuria.—If serum albumin is persistently present, found by nitric acid and boiling, and when deposited by the boiling test makes a layer as thick as half of the column of fluid, it indicates Bright's disease. It appears transiently in febrile diseases, blood-poisonings, pregnancy, epilepsy. It is found in poisoning from lead and arsenic. Traces sometimes appear in adolescents from trivial causes. Fatigue from prolonged

marches, or from long bicycle runs, or after the strain of athletic games, may cause disturbance of normal cell activity with transient appearance of albuminuria and even tube-casts from the kidney. To make the diagnosis of Bright's disease conclusive, when only small amounts are present, tube-casts must also be found, or some of the general symptoms, such as anemia, cardiac hypertrophy, dropsy, and characteristic eye-lesions.

MUCIN (Nucleo-albumin).

68. It is not surprising that the urine flowing over a mucous membrane should contain even in health this constituent of mucus. Usually only a trace can be discovered by coagulating with acetic acid or trichloracetic acid. In catarrhal states of the urinary passages it is present in larger amount. Recently it has been stated that nucleo-albumin is mucoid but not mucin. Its importance depends upon the fact that it is often confounded with serum albumin when tests requiring the organic acids—citric, acetic, and picric—are employed. The differentiation is made certain by the boiling test, which does not coagulate any protein but serum albumin.

ALBUMOSURIA (Peptonuria).

69. Albumose, which is a protein allied to albumin and peptone, is revealed by the following reactions: (1) The urine acidulated by *acetic* acid and heated, at first becomes cloudy, but clears up when boiled, the cloudiness returning when cold. (2) After performing the *boiling and nitric acid* test with a negative result, a cloud appears on cooling which again clears up by heat. (3) The

acetic ferrocyanid test having produced a cloudiness, which is removed by heat, the return of the cloud when the urine is again cold. (4) Saturation of the sample with crystals of sodium chlorid causes a cloud which dissolves by acetic acid and heat and reappears when cold; whereas, if albumin is present, the cloud persists.

As albumin usually accompanies it, it may be necessary to separate them by the following method: Put in a test-tube equal quantities of urine and a saturated solution of common salt.

Acidulate with acetic acid, boil and filter while hot. The return of the cloud as the hot filtrate cools proves that albumose is present.

Import.—Albumose is a constitutent of the seminal fluid, and sometimes may be accounted for by the presence of semen.

It has been found also in the urine in different diseases, associated with internal suppuration, in osteomalacia, multiple myeloma, and intestinal ulcer.

HEMATURIA.

If the blood of hematuria is derived from the *kidney*, it is well mixed with the urine, to which it imparts a brownish smoky appearance, and microscopic tube-casts can usually be found. If from the *ureter*, large vermicular clots may be found. If from the *bladder*, the blood is usually abundant and bright red, with stringy clots.

70. *Microscopic Appearances.*—The most satisfactory test for blood is the presence of the peculiar corpuscles. These corpuscles in ordinary acid urine retain their

biconcave shape for several days. (Fig. 23.) If the urine is dense, they become crenated. (Fig. 22.) When the urine is alkaline from ammonia, as in cystitis, the red corpuscles are shriveled and accompanied by numerous leukocytes. Urine containing blood is always albuminous, and if tube-casts are found, the albumin may be considered in excess of what can be accounted for by the blood alone.

HEMOGLOBINURIA.

When the blood-coloring principle hemoglobin is excreted in the urine, diffused and not corpuscular, other tests than the microscopic must be resorted to. These tests indicate hemoglobin, either with or without the corpuscles. The microscope will serve to exclude the corpuscular element.

71. The spectroscope may be applied to a feebly acid specimen, when the double band of oxyhemoglobin is seen if the blood is carefully diluted. By cautiously adding ammonium sulphid the spectrum changes to that of reduced hemoglobin.

72. *Heat Test.*—To a suspected sample add a drop of acetic acid, and boil. A red or brown coagulum indicates hemoglobin.

73. *Almen's Test.*—To suspected urine add a few drops of tincture guaiac, freshly made. A white or greenish turbidity ensues. Shake well and add a few drops of solution of hydrogen peroxid. Hemoglobin will slowly change the color of the precipitate to blue. Another method is to mix the tincture of guaiac with hydrogen

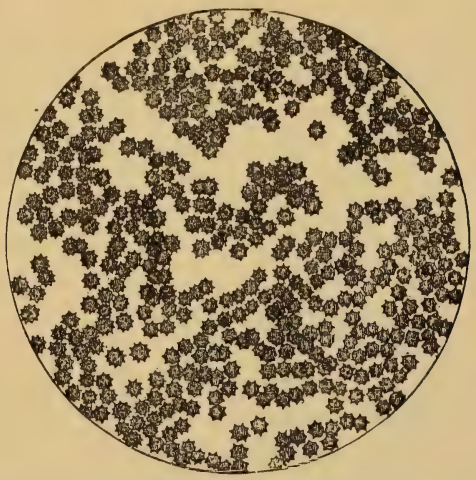

FIG. 22.—Crenated Red Blood-corpuscles in the Urine. ×350.

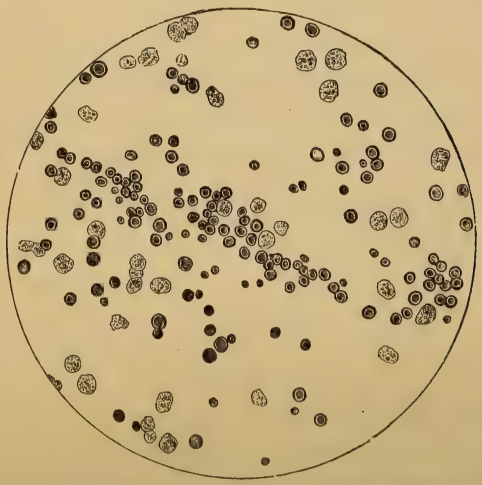

FIG. 23.—Colored and (*a*) Colorless Blood-corpuscles of Various Forms.

peroxid in equal parts and pour it gently down the inclined tube so as to form a top layer upon the urine. A white belt gradually turning blue indicates blood.

The mixture of bloody urine and guaiac may be dropped on a piece of sodium perborate on a white plate; it turns blue.

As saliva or potassium iodid produce an olive green color which masks the blue, these must be excluded before a positive conclusion can be reached.

74. *Heller's Test.*—Enough potassium hydrate is added to make the urine alkaline. On boiling, the phosphates separate, and, subsiding, carry down the hematin as a flaky red or brown precipitate. In healthy urine the precipitate will be white.

After the administration of santonin, chrysarobin, rhubarb, or senna, the urine contains a yellow principle which is reddened by the potassium hydrate and precipitated with the phosphates. To distinguish this from hematin the phosphatic deposit is filtered off and enough acetic acid must be added to it to neutralize the alkali. This clears up all the red precipitate except hematin, which fades slowly in the air. By this means oxyhemoglobin can be detected in a dilution of 1 to 4000.

Import.—Hematuria indicates hemorrhage from some part of the genito-urinary tract.

Hemoglobinuria occurs in various diseases, attended by "a dissolved state of the blood," as pyemia, scurvy, typhus, purpura, poisoning from hydrogen arsenid, phosphorus, carbolic acid, chloral, potassium chlorate. There is a periodic form, the pathology of which is obscure.

PYURIA.

As pus is an albuminous fluid, its presence will cause the urine to respond to the tests for albumin. In addition, it contains white corpuscles, which are spheric, granular, and opaque. (Fig. 7.) The mucus-corpuscle

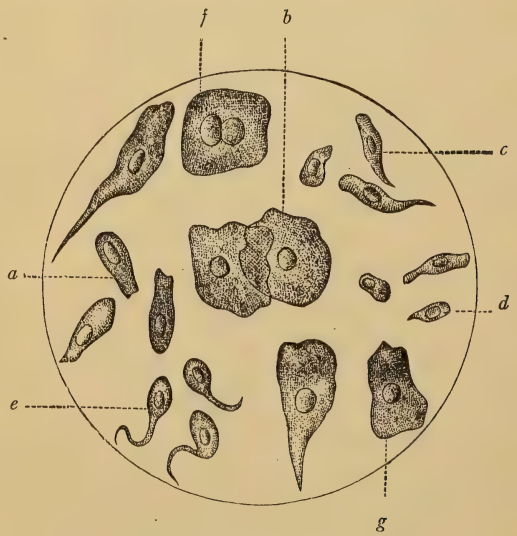

FIG. 24.—*a*. Epithelium from the human urethra. *b*. Vagina. *c*. Prostate. *d*. Cowper's glands. *e*. Littre's glands. *f*. Female urethra. *g*. Bladder.

is indistinguishable from it, but mucin will not form a white belt at the line of contact with the cold nitric acid test for albumin.

75. *Donne's Test.*—Let the suspected urine stand several hours for the pus to settle, and decant the clearer part. Into the deposit put a piece of potassium hydrate

FIG. 25.—*a*. Blood-cast. *b*. Granular cast with Epithelial cells embedded *c*. Amyloid cast.

FIG. 26.—Hyaline Casts.

and stir with a glass rod; pus will grow thick, tough, and gelatinous, while mucus will form flakes and become thin.

Import.—A sudden appearance of pus in the urine would point to the opening of an abscess. Usually, pyuria is an indication of catarrh or inflammation of some part of the genito-urinary tract.

EPITHELIUM.

The mucous membrane of any part of the genito-urinary passages may shed its cells into the urine. While there is a great variety in form, it is usually not difficult to distinguish renal epithelium from that of *extrarenal* origin. The accompanying figure (25) will give an idea of the microscopic appearance of the tessellated, the columnar, and the transitional cells that appear at times from different parts external to the kidney.

76. *Free renal epithelium* is spheric, granular, nucleated, with a very faint wall or no wall whatever. Its character is easily made out by the fact that careful search will always reveal renal tube-casts in the same sample.

TUBE-CASTS.

When either albuminuria or hematuria attends disease of the kidney structure, the deposit in the urine will be found to contain microscopic casts of the tubules. They may be found in cases in which neither albumin nor blood can be detected by ordinary testing. Sometimes they are made of coagulated fibrin, sometimes of mucoid exuda-

tion, sometimes of the plastic material of broken-down epithelial cells.

77. 1. *Epithelial casts* are composed of plastic matter with cells embedded. They mark the process of desquamation. (Fig. 25, *b*.)

2. *Hyaline casts* are either transparent, soft, and very delicate in outline, or transparent, well-defined, and brittle.

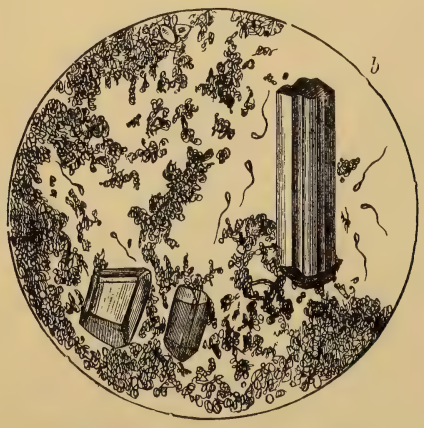

FIG. 27.—*a*. Spermatozoa. *o*. Amorphous calcium carbonate. *b*. Crystalline magnesium phosphate.

They are best seen with a flat mirror and side light. The former variety is called *mucous* (Fig. 26), and is not always an indication of nephritis; the latter, *waxy* (Fig. 25, *c*), is evidence of serious nephritis. Temporarily, hyaline casts and albuminuria are found as the result of excessive exertion in long marches, bicycle runs and athletic games. If not due to kidney disease they soon

disappear from the urine when a more moderate physical regimen is adopted.

3. *Granular casts* are opaque and yellowish. They are mucoid or waxy or cellular material, which has undergone a granular change. They often contain cells and fat globules. (Fig. 25, *b*.)

4. *Fatty casts* are those which carry free fat globules along with the coagulated matter. They are usually considered proof of fatty degeneration of the kidney.

5. *Blood-casts* are composed of coagulated blood with corpuscles embedded. (Fig. 25, *a*.) They show that the hematuria originates in the secreting structure of the kidney.

SPERMATURIA.

78. The presence of semen produces a transient show of proteins which give the reaction of mucin or albumose and may be mistaken for albumin. The characteristic elements are the spermatozoa. These may be present in the urine voided after coitus or an involuntary nocturnal emission of semen or be an indication of pathological spermatorrhea. They form a viscid, mucus-like deposit, which, under the microscope, is seen to be composed of ciliated bodies, like a tadpole, 1/600 of an inch long. In the urine they are motionless and resist decomposition for days.

CHYLURIA.

79. Under very rare conditions the urine contains chyle. It then presents the appearance of milk forming a cream at the surface, and owing to the fibrin of the chyle

it may coagulate spontaneously like a white jelly. It responds to the various tests for albumin. If agitated with ether, it clears up, the fatty matter is dissolved, and separates as a layer at the top. Under the microscope the fat appears in a molecular state as small, bright granules, soluble in a drop of ether. The same substances appear in the urine when the filaria sanguinis hominis is in the blood. In cases of feigned disease it may be necessary to see the urine passed in order to prove that milk was not added.

MICRO-ORGANISMS.

After emission, the urine may become the medium for the development of many low forms of life proceeding from germs that get into it from the urethra or from the air or from the containing vessels. Various *molds* and the *yeast fungus* (*Saccharomyces cerevisiæ*) are commonly met with in putrid or saccharine urine after several days. *Sarcinæ* in quantities have been found in urine at the time of discharge, probably produced in the bladder. Local symptoms are supposed to be due to their growth. These vegetations appear in microscopic tubes composed of distinct particles.

Bacteria not only cause the ordinary decomposition of stale urine outside, but sometimes set up like processes inside, the bladder. It is likely that the micrococcus ureæ gets access to the urine in the urinary passages by means of dirty instruments, such as catheters and sounds. They may steal down the urethra in the inflammatory products of old gonorrhea. If, now, the urine is detained in the

bladder, these organisms set up the ammoniacal fermentation that induces cystitis. By the use of the centrifuge a sediment of bacteria can be readily collected. They must be studied by high-power lenses after drying and staining on the cover-glass. The presence of pathogenic bacteria in significant quantities would denote the partic-

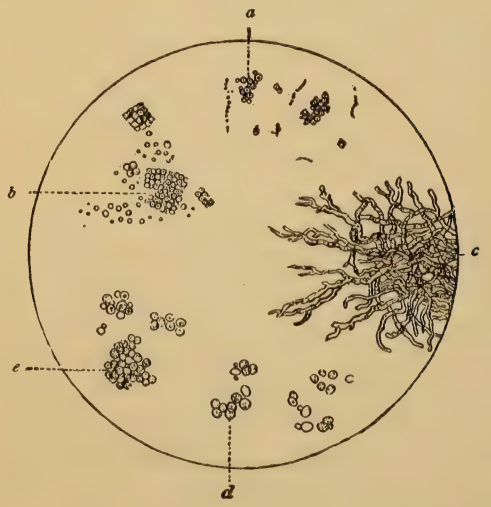

FIG. 28.—*a*. Micrococci in short chains and groups. *b*. Sarcinæ. *c*. Fungi from acid fermentation. *d*. Yeast cells from diabetic urine. *e*. Mycelium of a fungus.

ular disease to which they pertain, such as tuberculosis, erysipelatous nephritis, pyemia, endocarditis, gonorrhea.

80. *Ehrlich's Diazo-reaction.*—This test for certain "aromatic substances" which give color reactions to diazosulphobenzol requires two solutions—one containing sulphanilic acid, 1 gm. dissolved in 25 c.c. of hydro-

chloric acid made up to 500 c.c. of water, and another containing 1 gm. of sodium nitrite in 200 c.c. of water. These are not mixed until the test is to be performed. The test mixture is made with 1 part of the sodium nitrite solution and 100 parts of the sulphanilic acid solution. An equal part of urine is well shaken with the test mixture and then overlaid with ammonium hydrate. The significant substances will cause a carmin or garnet-red ring at the juncture, while normal urine is yellow. When shaken a foam is formed which is red, and the solution poured into a white basin full of clear water gives it a salmon color.

Practical Import.—This reaction is commonly present in typhoid fever, is rarely absent in measles, and has been frequently observed in tubercular meningitis.

Fallacy.—Salicylic acid and other aromatic derivatives give a like reaction. All coal tar derivatives must be excluded before making the test.

81. *Lead in the Urine Due to Plumbism.*—Owing to the wide distribution of lead, a trace may be present in the reagents or be derived from the glaze of the evaporating dish. To make sure, it is best to make a parallel experiment using the same process with normal urine.

To two liters of urine add 50 c.c. of nitric acid and evaporate down to 100 c.c. Transfer to a small dish, preferably of platinum and heat to a thick paste over a naked flame. Raise the heat until deflagration ends in a white residue. It may be necessary to cool off, moisten again with nitric acid and again heat until the residue is white.

To the cool residue add 20 c.c. of a 10 per cent. solution of acetic acid and boil. Filter and divide the filtrate

into two portions. Into one, drop a small crystal of potassium iodid; lead will cause a yellow appearance next the crystal. To the other portion in a test-tube add 1 c.c. of a strong solution of hydrogen sulphid; lead will cause a darkening.

Practical Import.—The discovery of lead in the urine is conclusive of its presence in the system.

82. *Mercury in the Urine.*—It is best to operate upon urine concentrated by evaporation over a water-bath, but this is not essential. Powdered zinc 10 grams (160 grains are boiled for ten minutes in 200 c.c. (6 fluidounces) of urine. The zinc having amalgamated with the mercury is separated by filtration, washed and dried in a water oven. A well dried slender test-tube receives the zinc powder. With the Bunsen flame the tube is softened an inch or two above the zinc and constricted. A loose plug of asbestos wool is placed at the constriction and upon it is laid a crystal of iodin, pin-head in size. Strong heat is applied to the zinc and at the time of volatilization of the mercury the flame is changed to the iodin which vaporizes in a moment and with the mercury vapor forms a deposit on the glass of red mercuric iodid.

Besides the different constituents of urine, healthy and morbid, which are seen in the deposits, extraneous substances are sometimes found. Some of these microscopic bodies, such as hair, or fibers of cotton and linen, may be mistaken for tube-casts. A dirty vessel is often responsible for the presence of oil, starch, wood, etc. *Healthy urine* may form, at or near the bottom, a light cloud of epithelial debris suspended in mucus. But if on emission an *acid* urine is *turbid* throughout, excess of mucus or pus

or blood is present. If an *alkaline* urine is *turbid*, then the precipitated phosphates may be the cause.

URINARY DEPOSITS.

83. A *specimen* may be obtained for examination by allowing the urine to settle, and then by means of a pipet a drop or two of the sediment is conveyed to the glass slide inspected with a low power first for suspicious objects and then covered with a thin glass disc for studying the details.

Examine with a 1/4 inch objective, or any combination giving a power of 200 to 300 diameters.

If *amorphous*, it may be urates or phosphates.

1. Put a few drops of the deposit into a test-tube with some urine and *warm* it; the urates will dissolve, the phosphates not.
2. To another portion add any acid, such as nitric or acetic; the earthy phosphates will dissolve.

If minute *globular particles* with bright centers, it may be milk, chyle, or fat.

Shake some of the urine with ether; if chylous, its milky appearance will disappear and the fat separate as a top layer.

If *crystalline*, it may contain one or more of the substances already described and figured: Uric acid (Figs. 9–11), calcium oxalate (Fig. 13), triple phosphate (Fig. 7), calcium phosphate, cystin (Fig. 15), leucin, and tyrosin (Fig. 16).

If *organized* bodies, they may be epithelial cells (Fig. 25, *b*), mucus or pus (Fig. 7, *b*), blood (Figs. 23, 22),

spermatozoa (Fig. 27, *a*), yeast fungus, mold, sarcinæ, bacteria (Fig. 28).

If a microscope is not at hand, the nature of an unorganized deposit may be determined by the following method:

84. *Rough Tests for Common Unorganized Deposits:*

1. Warm the deposit with some urine.

It dissolves = urates.

It does not dissolve = phosphates or calcium oxalate or uric acid.

2. Warm a fresh portion with acetic acid.

It dissolves = phosphates.

It does not dissolve = calcium oxalate or uric acid.

Warm the undissolved portion with hydrochloric acid.

It dissolves = calcium oxalate.

It does not dissolve = uric acid.

URINARY CONCRETIONS.

About 80 in 100 of all urinary concretions are made of *uric acid and urates*. Next in frequency are the *calcium oxalate*, or mulberry, calculi. The rarer primary constituents are *blood concretions, cystin, xanthin, calcium phosphate, calcium carbonate*. Secondary to any of these there may appear at the last stage in the history of a calculus a deposit of *mixed phosphates*. These form a white layer, precipitated upon the calculus as a result of ammoniacal decomposition in the urine.

As two or more deposits of different compositions may alternate in the formation, in *examining* a concretion it should be sawed through the middle, so as to expose its

concentric layers. A small portion of each distinct layer should be examined by the following method:

85. CASUAL ANALYSIS OF CONCRETION.

First burn a portion of the powder on platinum foil in a Bunsen burner or blowpipe flame.

A. *It chars greatly, leaving but little ash* = uric acid, urates, cystin, xanthin, blood.

It gives murexid reaction (see p. 29) = uric acid or urates.

It dissolves in boiling water = urates.

It does not dissolve in boiling water = uric acid.

Cystin and xanthin are very rare; the first can be recognized by test on page 36.

B. *It chars very slightly, leaving very much ash* = phosphates, oxalate, carbonate of calcium.

1. Treat a fresh portion with dilute hydrochloric acid.

It is soluble with effervescence = carbonates are present.

It is soluble without effervescence = phosphates or oxalate calcium.

2. Treat with acetic acid.

If *it is soluble without effervescence*, then it is phosphatic;

it fuses into a bead on platinum foil = mixed phosphates;

it does not fuse = calcium phosphate.

If *it is insoluble*, then it is calcium oxalate, and when calcined on platinum, leaves an ash that turns red litmus blue, or effervesces with HCl.

PART II.

EXAMINATION

OF THE

GASTRIC CONTENTS.

By the term gastric contents, used in this manual, is meant the material found in the stomach one hour after a simple *test-meal* has been administered. At this time normal gastric digestion is about at its height. The meal to be referred to hereafter represents proteins and carbohydrates with the usual inorganic constituents of food. Before the expiration of one hour the food has been altered by digestion. The salivary ferment *ptyalin* has converted some of the starchy material into dextrin and glucose. Part of the proteins have been converted to peptone. Part of the glucose has undergone a fermentation by the bacteria which generate *lactic acid.*

Immediately upon the reception of food the secretion of gastric juice begins. This is a clear, pale yellow, thin fluid, acid in reaction, holding in solution about one per cent. of solids mostly organic in character. The *quantity* secreted daily is very large—while we have no exact data, it has been estimated to average from 4 to 14 pints. Its *specific gravity* varies between 1001 and 1010.

COMPOSITION OF GASTRIC JUICE.

	Per Cent.
Water	99.44
Solids, as tabulated below	0.56
Organic substances (pepsin and peptones)	0.32
Free hydrochloric acid	0.25
Sodium, potassium, and calcium chlorids	0.21
Calcium, magnesium, and ferric phosphates	0.02

86. PLAN FOR CLINICAL EXAMINATION.

(*a*) Withdraw the gastric contents one hour after the administration of the "test breakfast" (§ 88), noting if additional water was necessary for evacuation.

(*b*) Note the color, odor, and other physical characters.

(*c*) Filter one-half of the contents.

(*d*) Start the digestion test for pepsin (§ 90).

(*e*) Determine the total acidity with sodium hydrate (§ 109).

(*f*) Determine loosely combined acids.

(*g*) Determine free hydrochloric acid.

(*h*) Test for the organic acids.

(*i*) If the appearance warrants it, test for bile (§ 2) and blood (§ 73).

REAGENTS REQUIRED.

Congo-red paper.
Gunzburg's or Boas' solution.
Solution carbolic acid, four per cent.
Solution neutral ferric chlorid (§ 101)
Tropæolin O O.
Egg-albumin.
Phenolphthalein, one per cent. alc.

Hydrochloric acid.
Solution of sodium hydrate N/10 (decinormal). This solution should be carefully made and titrated against a standard solution of pure crystallized oxalic acid.
Alizarin monosulphonate of sodium 1 per cent. aqueous solution.
Toepfer's solution, dimethylamido-azo-benzol 0.5 per cent. alcoholic solution.
Lactic acid.
Pepsin.

87. *Artificial fluids* for practice are easily made by adding to water small amounts of hydrochloric acid, or lactic, acetic and butyric acids.

Gastric juice and digested contents are prepared artificially by dissolving peptone of the shops in water, with common sirup for glucose, and minute quantities of the acids hydrochloric, lactic and butyric. Liquid pepsin and rennet may also be added when the digestive power is to be tested.

THE SAMPLE.

The specimen to be examined should contain those most important constituents of the gastric juice—pepsin and hydrochloric acid. After taking food, for the first three-quarters of an hour the hydrochloric acid present is not free but combined with proteid substances, and does not interfere seriously with the amyloid digestion by the alkaline saliva. If the reaction at this first period should be acid, the acidity is due to the organic acids—lactic, butyric, and acetic—produced by fermentative changes in the food. After an hour only a trace of lactic acid should be found, while the amount of hydrochloric acid increases for two or three hours. The sample for clinical examination is usually obtained after a test breakfast.

88. *Ewald's Test Breakfast.*—This consists of 9 drams (35 gms.) of dry bread (an ordinary roll of about this weight) and 10 fluidounces (300 c.c.) of warm water or very weak tea without milk or sugar. In an hour after taking this will be liquefied by digestion and can be expressed through the tube. The stomach-tube is made of soft, smooth, flexible rubber, long enough to reach the stomach and leave several feet free outside the mouth. It should have a large free bore and several smoothly rounded windows near the end that enters the stomach. The patient sits erect in a chair with his head thrown back. After wetting the tube in warm water, it is passed back to the pharynx and held there in spite of gagging until the patient makes an effort at swallowing. By gentle pressure it can then be readily passed into the stomach. Sometimes the gastric contents are evacuated at once without effort. Pressure over the abdomen while the patient coughs will almost always force them out. At times it is necessary to wash out the stomach by inserting a funnel into the outer end of the tube and filling tube and funnel with warm water of known quantity. Then to make a siphon before all the water has run from the funnel, the tube is lowered into a vessel on the floor. If 10 fluidounces (300 c.c.) of water or tea have been given, about 1 1/2 fluidounces (40 c.c.) of fluid should be obtained by the tube. By filtration a clear solution can be had for the application of the tests.

89. In some cases, such as suspected ulcer, and when the patient is highly nervous, it is necessary to make a small amount suffice for the necessary tests. To secure this, *Einhorn's little silver bucket* is swallowed an hour

after the test breakfast. After five minutes the bucket is withdrawn by a cord. If resistance is felt when the bucket gets as high as the larynx, a deep expiration will remove it.

PEPSIN.

Pepsin is the ferment which changes proteid substances into peptones in an acid medium. Normally, it is associated with hydrochloric acid; but the lactic acid found in the stomach, even when hydrochloric acid is absent, will aid it to dissolve the proteids. To detect its presence the following procedure is used:

90. *Test for Pepsin.*—First boil an egg for four minutes. Having cut the white part into thin flakes, punch out small discs with a cork-borer or a glass tube. Such pieces as are not wanted can be kept indefinitely in glycerin and washed before using. Into a test-tube or watch-glass containing two drams or more of filtered gastric contents, put two drops of hydrochloric acid and a disc of egg-white. Stand asidefor a half-hour or more at a temperature of 104° F. (40° C.) If pepsin is present, the disc will be made rough and smaller by partial solution. For practice the student can make an artificial gastric juice by dissolving 1 1/2 grs. of pepsin in a fluid-ounce of water and adding 5 drops of hydrochloric acid.

91. *Estimation of Pepsin Strength.*—Cut the coagulated egg-white into pieces 1/25 of an inch (1 mm.) thick, and punch out discs 2/5 of an inch (10 mm.) in diameter. Into each of two test-tubes put two discs and 2 1/2 drams (10 c.c.) of filtered gastric contents. To one of these tubes add two drops of strong hydrochloric acid, and then

stand both in an oven heated by a thermostat to 100° F. (40° C.). If pepsin and acid are present in normal amount, the discs in both tubes will dissolve in one to two hours. If the acid is deficient, solution will occur in the tube only to which the hydrochloric acid was added.

Clinical Import.—Pepsin is very rarely absent, even in serious disease of the stomach. Failure in gastric digestion is seldom due to the lack of it. If the flakes of egg-white digest without adding hydrochloric acid, it does not prove a normal state of things, as digestion is possible by the presence of lactic acid alone, though the condition is not a healthy one. It is of the first importance to prove the presence of hydrochloric acid by tests to be given hereafter.

PEPTONE.

92. Peptone may be recognized by the test (see § 69, page 67). It is seldom of any value to carry the investigation further, but, if desired, albumin may be detected in a fresh sample by a precipitate formed on boiling; when this is separated by filtration, a precipitate obtained from the filtrate by adding nitric acid denotes propeptone; after a second filtration, the addition of solution of tannin will precipitate peptone.

HYDROCHLORIC ACID.

In the gastric juice free hydrochloric acid is present 0.25 per cent. One hour after a test breakfast, if hydrochloric acid is detected, the amount of lactic acid is so

small as to be safely disregarded in causal analysis for clinical purposes. The hydrochloric acid exists partly in a loose combination with proteins and partly free. In various chronic diseases of the stomach a chemic examination of the gastric contents must be made to judge of the extent and nature of the morbid mischief. The main point is the determination of the presence of hydrochloric acid and its amount, free and combined.

93. *The Acid Reaction.*—To detect the presence of free hydrochloric acid, litmus paper will not serve, as it is affected by acid salts and by the organic acids—lactic, butyric, and acetic. It has been found convenient to use paper colored with certain anilin dyes (Congo-red and tropæolin are the best) which react to minute quantities of free hydrochloric acid, but which are unaffected by acid salts and by the organic acids—lactic, butyric, and acetic —only when present in 0.5 per cent.—an amount never found in the gastric contents now being studied. The most delicate reaction for distinguishing hydrochloric acid from the organic acids is that given by Toepfer's solution of dimethyl-amido-azo-benzol.

Detection of Free Hydrochloric Acid.

94. *Toepfer's Test.*—On a white plate or porcelain dish place one drop of the gastric contents filtered or unfiltered and touch it with a drop of Toepfer's 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol. Free hydrochloric acid as little as 0.002 per cent. changes the yellow color of the indicator to a cherry-red. The organic acids of the test meal do not influence it.

95. *Congo-red Test.*—Take a piece of Congo-red paper and put upon it a drop of the gastric contents. A dark blue spot will appear if free hydrochloric acid is present as much as 0.05 per cent. A violet spot or a blue ring only around the wet place signifies some free acid, either a trace of hydrochloric acid or some organic acid.

96. *Tropœolin (Dimethyl-orange) Test.*—A positive reaction with this test means that hydrochloric acid is present at least 0.02 per cent.; a negative reaction would lead to the other more delicate test given later on. Three drops of the yellow-brown test solution (saturated alcoholic) are allowed to flow over a dish. As much gastric fluid is added and spread in the same way and gentle heat is applied. Blue or lilac stripes are caused by hydrochloric acid, but not by organic acids. If the latter are present in large amount they may turn brown but not blue.

Another method uses prepared paper: Have on hand saturated alcoholic solution of pure tropæolin (O O). Shortly before use, immerse in this solution some pieces of white filtering paper and dry them. (This paper loses its sensitiveness on keeping.) Wet the prepared paper with a drop of the gastric contents. If hydrochloric acid is present to 0.02 per cent., a dark reddish-brown spot appears, which will change to lilac when gently heated on a watch-glass held by the fingers over a small flame. The organic acids as found in the gastric contents do not have any effect. In larger amounts they make a faint brown stain which does not show a lilac color when heated.

97. *Phloroglucin Vanillin (Gunzburg's) Test.*—It is

best to make the reagent fresh. It will serve for a while if kept in dark-colored bottles. Take of—

Phloroglucin,	2 parts (30 grs.)
Vanillin,	1 part (15 grs.)
Absolute alcohol,	30 parts by weight
Or—	(1 fluidounce)

Alcohol, 80 per cent, 100 parts by measure. Mix. Make a clear, pale-yellow fluid.

98. *Boas' resorcin solution* is a reagent of almost equal delicacy, has greater stability, and is cheaper than Gunzburg's. To make it take of—

Resorcin, pure,	5 gms.
White sugar,	3 gms.
Dilute alcohol,	100 c.c .

These two tests are applicable to free mineral acids only, being unaffected by the amount of organic acids found in the gastric contents. The method is the same for both.

99. *Method for Gunzburg's or Boas' Tests.*—Mix upon a watch-crystal or porcelain dish about five drops of the test solution and three of the gastric contents, either filtered or unfiltered. Gently heat the dish held in the hand by cautiously moving it over a small flame, and if free hydrochloric acid is present to 0.005 per cent., a bright red ring will form at the margin as the mixture dries. When several samples are to be tested, or when the reagent is used to indicate the end of a reaction, it is more convenient to put one cubic centimeter of the reagent on a flat porcelain dish and dry it by constant motion, so as to spread it in a thin layer. The heat should not be above 110° C., or charring will ensue. A

glass rod carrying a drop of the gastric fluid is touched to the prepared reagent and the dish cautiously dried. A red ring will appear if so much as 0.005 of free hydrochloric acid is present.

Precaution.—The proteins in the gastric contents, if evaporated and dried with a strong heat, may show a deep red color at the center. To avoid this fallacy, the evaporation must be done slowly or at a gentle heat. Hydrochloric acid will then cause a red rolor where drying first occurs, at the edges, or in streaks.

100. *Approximate Estimate.*—By diluting a portion of the gastric contents to 1/2 or 1/5 or 1/10 or 1/20 until no reaction occurs on the dish prepared with dried Gunzburg's reagent, the amount of free hydrochloric acid can be estimated. As the limit of the reaction is about 0.005 per cent., a red color with the 1/10 dilution would indicate 0.05 per cent. or more; with the 1/20 dilution it would mean as much as 0.1 per cent.; with the 1/30 dilution as high as 0.15 per cent.; with the 1/40 as high as 0.2 per cent.; with 1/50 the amount of acid would be normal; with 1/60 the acidity would be in excess, that is, 0.3 per cent.

Clinical Import.—The *absence* of free hydrochloric acid in the contents taken one hour after the test breakfast denotes either such a disorganization of the gastric mucous membrane as is found in atrophy and in amyloid degeneration, or the dilatation which accompanies gastric cancer and chronic catarrh. The presence of hydrochloric acid does not speak with positive certainty against the diagnosis of gastric cancer. Collateral points must be considered before making a diagnosis, as there are several

possible conditions causing absence and several causing excess of hydrochloric acid.

DETECTION OF ORGANIC ACIDS.

101. *Lactic Acid by Ferric Chlorid Test.*—Make the reagent by diluting a few drops of ferric chlorid solution until its yellow color is almost gone. Having placed in a test-tube several inches of filtered gastric contents not yellow in color, add about half as much of the dilute ferric chlorid, looking for a canary-yellow color due to ferric lactate.

If hydrochloric acid has not been previously detected in a sample of the gastric contents, it is most probable that lactic acid will be found.

If the above test does not show it, the amount may not be great enough and it is necessary to separate and concentrate it by shaking with ether.

102. *Carboloferric or Uffelmann's Test.*—Prepare Uffelmann's reagent freshly by mixing one drop of a dilute solution of ferric chlorid (U. S. P.) with 2 1/2 fluidrams (10 c.c.) of a 4 per cent. solution of carbolic acid and 5 drams (20 c.c.) of water. Or, if desired, the following official preparation may be used:

℞. Liq. ferri chloridi, gtt. j.
Liq. acidi carbolici, gtt. iv.
Aqua, f℥ v. M.
Sig.—Uffelmann's reagent.

When first made, the reagent has an amethystine blue color.

Method.—Mix equal parts of Uffelmann's reagent and filtered gastric contents. If even so small an amount as

0.01 per cent. of lactic acid is present, the color changes to canary-yellow or greenish-yellow. By the other gastric acids the color is not changed to the same depth of yellow, though the blue color may be discharged by them if the very unusual amount of 0.3 per cent. is present.

Fallacies.—Other constituents of the gastric contents may give the same yellow color. These are glucose, alcohol, acid phosphates, butyric acid.

Lactic acid in the complex gastric contents sometimes turns the reagent brown or grayish white. In fact, the test is often of uncertain value.

In cases of doubt, to eliminate any chance of error, the lactic acid must be separated by shaking 2 1/2 drams (10 c.c.) of the gastric contents with 10 drams (40 c.c.) of ether for thirty minutes. The test is then applied to the liquid residue left when the ether has evaporated by first adding 1 c.c. of distilled water and then the Uffelmann's reagent. A lemon-yellow color is obtained in the presence of only 0.1 per cent. of lactic acid.

Clinical Import.—The small amount of lactic acid found in the early stages of normal digestion is explained by the fact that in the mouth are various bacteria which can cause the lactic fermentation in the carbohydrates. In the mouth starch is converted by ptyalin to glucose $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$ and the lactic acid ferments change the glucose to lactic acid $C_6H_{12}O_6 = 2C_3H_6O_3$. In the later stages of digestion this small amount has been absorbed and the fermentation stopped by the anti-fermenting powers of the hydrochloric acid. When the hydrochloric acid is absent from pathological causes like carcinoma, the proportion of lactic acid may rise as high

as 0.1 and 0.38 per cent. Large amounts like this may justify the diagnosis of gastric carcinoma. If there is retained any power of secreting hydrochloric acid, then even in carcinoma, the lactic acid may not be found.

103. ACETIC AND BUTYRIC ACIDS.—The residue obtained by evaporation of the ethereal extract above mentioned contains all the organic acids. If they are present, this residue will be an acid fluid showing the volatile acetic and butyric acids by their odor.

104. *Butyric Acid.*—In the very rare cases of stagnation of the gastric contents when fermentation produces this acid in the large amount of over 0.5 per cent., it changes Uffelmann's blue reagent to a red-brown. If a portion of the ethereal extract is diluted and a piece of calcium chlorid added, the light butyric acid will float like oil globules on the saline solution below. Lactic acid has fermented and produced the fatty acid in accordance with the equation:

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 4H.$$

105. *Acetic Acid.*—Having neutralized the ethereal extract by carefully adding sodium or potassium hydrate, add one drop of solution of ferric chlorid. Acetic acid will form deep red ferric acetate, which on boiling will precipitate as the brownish basic salt.

106. *Quantitative determination of organic acids* by Braun's method is performed on 10 c.c. of the gastric fluid which is exactly neutralized with decinormal solution of sodium hydrate, evaporate to dryness on a water bath, and the residue heated by ignition as long as it burns with a flame. The organic salts of sodium formed by

neutralizing are thus converted into sodium carbonate. The cooled residue of carbonates is extracted with boiling distilled water, filtered, and the amount of carbonates determined by titrating with decinormal HCl. The best results are obtained by first adding a measured excess of decinormal HCl, boiling off the CO_2, and determining the excess of acid with decinormal sodium hydrate, using phenolphthalein as indicator. The acidity due to organic acids in the 10 c.c. of gastric liquid is calculated by the difference between the amounts of acid and alkali used. It may be stated in terms of the amount of decinormal sodium hydroxid multiplied by 10 to give the per cent. If 3 c.c., then the acidity would be called 30 per cent. or 30 degrees.

Clinical Import.—The fatty acids, butyric and acetic, are formed under conditions essentially the same as those that favor the lactic acid fermentation. They are not present in the gastric contents after the test breakfast, in health, dilatation, atony, or inflammation of the stomach. Like lactic acid they are almost constantly present in carcinoma.

ACIDITY.

107. The hydrochloric acid is an antiferment and germicide for all the bacteria except those of anthrax and tubercle. When it has been detected in the gastric contents one hour after a test breakfast, by some of the tests above named, it is sometimes assumed that there is very little if any of the organic acids—lactic, acetic, and butyric—which are mainly products of fermentation. Conversely, to discover the organic acids by Uffelmann's test

is to establish the presumption against normal amounts of hydrochloric acid. The acidity of the small proportion of organic acids that may be present with hydrochloric acid is so small as to be a negligible quantity in some clinical studies. After ascertaining the presence of hydrochloric acid by Toepfer's or Boas' test, and the absence of lactic acid by Uffelmann's to determine the total acidity is practically to estimate the amount of hydrochloric acid present. The degree is expressed in terms of that acid.

108. *Preliminary Standardizing.*—Absolute accuracy is necessary in making the solution for acidimetry. This is controlled by titrating the soda against decinormal oxalic acid. Two solutions must be made (*a*) 4.5 grams of sodium hydroxid (by lime) in 900 c.c. of distilled water in a large bottle. More of the soda is dissolved than 1/10 its molecular weight (4.0) would call for, because some of the weight is certain to be due to the moisture attracted from the air. To insure accuracy titrate the soda solution with (*b*) 6.285 grams of oxalic acid dissolved in 1000 c.c. of water. Procedure: Put 10 c.c. of the oxalic acid solution (*b*) in a beaker, add two drops of phenolphthalein as indicator and from a filled buret run in the soda solution (*a*). If the soda solution is of standard strength it takes just 10 c.c. to produce the red color which does not become deeper after adding one more drop. If it takes less than 10 c.c. then the soda solution is too strong and distilled water must be added according to the formula

$$N = \frac{R \times d.}{c}$$

N is the number of c.c. of water to be added to the solution remaining; R is the total number of c.c. of soda solution

remaining after titration; d the difference between the number of c.c. of soda used and that required theoretically, and c the number of c.c. of soda consumed in the titration.

Thus if 9 c.c. of soda solution were used in neutralizing the 10 c.c. of acid $c=9$ and the soda was too strong by the difference; $d=10-9=1$. There will remain out of the 900 c.c. of soda solution $R=891$ c.c. and the calculation is made.

$$N=\frac{891\times 1}{9}=99.$$

All the soda left in the buret is put back into the bottle with 99 c.c. of distilled water. On repeating the test the 10 c.c. of acid solution will be exactly neutralized by 10 c.c. of soda solution and the theoretical standard is adjusted (1 c.c. contains 0.004 gram NaOH and will neutralize 0.00364 gram HCl).

109. *Toepfer's Method, Quantitative Estimation of the Acids Free and Loosely Combined.*—Having filtered the gastric contents measure 10 c.c. (if not enough use 5 c.c.) of the filtrate in each of three small dishes or beakers numbered respectively, No. 1, No. 2 and No. 3.

First Stage, "Total Acidity.—"To the contents of dish No. 1 add two drops of 1 per cent. alcoholic solution of phenolphthalein as indicator. A 25 c.c. buret is charged with the decinormal solution of sodium hydroxid, and a few drops at a time are run into the colorless liquid in the dish until a deep red color persists. If 10 c.c. of the gastric contents have been used and the amount of hydrochloric acid is normal, it will require from 4 to 5 c.c. of the sodium hydroxid solution to change the color. As

each cubic centimeter of this alkali solution will neutralize 0.00364 gm. of HCl; if 7 c.c. have been required, then the acidity is calculated thus: $7 \times 0.00364 = 0.02548$ of HCl. If 10 c.c. of gastric liquid were used, then to get percentage $0.02548 \times 10 = 0.2548$ per cent. of HCl. If 5 c.c. were used, then $0.02548 \times 20 = 0.50960$ per cent. of HCl.

Sometimes for clinical records the acidity is expressed by the number of cubic centimeters of N/10 sodium hydroxid which is required to neutralize 100 c.c. of the stomach liquid. An acidity of 70 per cent. would then mean that 100 c.c. of the gastric liquid required 70 c.c. of N/10 sodium hydroxid to neutralize it.

Some of the acid reported by the "total acidity" when phenolphthalein is used as indicator, is in reality not free but loosely combined with proteids. When so desired, the free hydrochloric acid, the acid proteids and the organic acids may be estimated by Toepfer's method continued with dishes No. 2 and No. 3.

110. *Second Stage, for "Acids Not Loosely Combined."*—The indicator used in dish No. 2 is three drops of a 1 per cent. aqueous solution of alizarin monosulphonate of sodium. The yellow color is maintained by free acids but not by acid proteins. When tested in distilled water the color is yellow or reddish, in a weak solution of disodium phosphate it is reddish with a tint of violet. A weak solution of sodium carbonate gives the pure violet reaction desired. Having added three drops of alizarin solution to the 10 c.c. of gastric contents in dish No. 2, run in, drop by drop, the decinormal sodium hydroxid of the buret until a clear violet appears. If 5 c.c. of N/10

sodium hydroxid were required for 10 c.c. of gastric fluid then the *free acids* are represented by their equivalence to 50 per cent. of sodium hydroxid. The difference between the results with dishes No. 1 and No. 2 would represent the quantity of sodium hydroxid solution equal to the acid proteids in 10 c.c. of gastric contents thus:

7 c.c. were needed for the "total acidity" dish No. 1.
5 c.c. were needed for the "acids not loosely combined" dish No. 2.
2 c.c. represents the "loosely combined acid."

111. *Third Stage, for "Free HCl."*—The indicator for this stage is 0.5 per cent. alcoholic solution of dimethyl-amido-azo-benzol. Its natural yellow is changed to red by free hydrochloric but not by the organic acids or acid proteids.

Having added three drops of the yellow indicator to the 10 c.c. of gastric contents in dish No. 3 a cherry-red color denotes *free hydrochloric* acid. If the normal yellow is retained there is no *free HCl.* Run in the decinormal sodium hydroxid from the buret drop by drop until the cherry-red disappears and the normal greenish-yellow is restored. If 4 c.c. were required to neutralize the 10 c.c. of gastric contents then 4×10 c.c. $=40$ c.c. gives the sodium hydroxid equivalent to 100 c.c. of gastric contents. To get free HCl by weight, then 40 c.c. $\times$ $0.00364 = 0.14560$ grams free HCl in 100 of gastric contents.

112. *Fourth Stage for Organic Acids and Acid Salts.*—By subtracting the number of c.c. of the decinormal sodium hydroxid required for dish No. 3 from that required for dish No. 2, the remainder represents the c.c.

of the decinormal sodium hydroxid required for the organic acids and acid salts in 10 c.c. of gastric contents:

5 c.c. were needed for dish No. 2, "acids not loosely combined.' '
4 c.c. were needed for dish No. 3, "free hydrochloric acid."
1 c.c. the equivalent of the "organic acids and acid salts."

113. *Clinical Report of Analysis.*—In the particular gastric contents analyzed by the four stages given above the quantities of decinormal sodium hydroxid required for 100 c.c. may be tabulated as follows:

Total acidity equivalent to,	70 c.c.
Acids not loosely combined,	50 c.c.
Acid proteins,	20 c.c.
Free hydrochloric acid,	40 c.c.
Organic acids and acid salts,	10 c.c.

The percentage by weight of free hydrochloric acid was 0.14560.

114. CLINICAL IMPORT OF FREE HYDROCHLORIC ACID.

Euchlorhydria is the secretion of hydrochloric acid in normal amounts (0.1 to 0.2 per cent.). Though the acidity may be normal in functional dyspepsias, it is never so in chronic inflammation of stomach.

Hypochlorhydria is a diminished secretion of hydrochloric acid (less than 0.1 per cent.). This condition attends disease which impairs the structure of the gastric glands, as dilation, cancer, atony, chronic gastritis, and very rarely in gastric ulcer.

Anachlorhydria is absence of hydrochloric acid, found in long-continued chronic gastritis, even in nervous dyspepsia, such as hysterical anacidity. The acid may

be absent in gastric cancer, but is sometimes present even to excess when the cancer is a sequel to ulcer.

Hyperchlorhydria is increased secretion of hydrochloric acid, found in nervous dyspepsia, gastric ulcer, and possibly in carcinoma growing upon an old ulcer.

115. MOTOR POWER OF THE STOMACH.

Ewald's salol test is the best for determining the sufficiency of the stomach in propelling its contents onward to the duodenum. Salol (phenol salicylate) in an hour passes unaffected by the acid contents of the stomach into the bowels, where it encounters the alkaline pancreatic fluid and is split into salicylic acid and phenol. These are absorbed and appear directly in the urine.

Method.—Give 10 or 15 grs. of salol in a capsule or coated pill. In an hour test the urine for salicylic acid by wetting a piece of filter paper with urine and touching the moistened paper with a drop of a 10 per cent. solution of ferric chlorid. A trace of salicylic acid is sufficient to develop a violet ring around the drop. A more conclusive result is obtained if the urine is examined after thirty hours. The salicylic acid should all have been absorbed and eliminated and therefore absent from the urine before that time. If the violet reaction should appear at this time, there is proof of sluggish action of the stomach.

PART III.

EXAMINATION

FOR

COMMON POISONS.

CLASSIFICATION OF POISONS.

The following simple arrangement of commonly occurring poisons is adopted as suited for the preliminary tests of the practitioner of medicine:

A. Corrosive Acids. *Nitric*, HNO_3; *Hydrochloric*, HCl; *Sulphuric*, H_2SO_4; *Oxalic*, $H_2C_2O_4$.

B. Corrosive Alkalies. *Potassa*, KHO; *Soda*, NaHO; *Ammonia*, NH_4HO.

C. Volatile Matters. *Carbolic Acid*, C_6H_5OH; *Hydrocyanic Acid*, HCN; *Phosphorus*, P; Chloroform, $CHCl_3$; Chloral, C_2HCl_3O; Alcohol, C_2H_5HO.

D. Metallic Irritants, subdivided into

1. Those precipitated *yellow* or *orange* from an acid solution by hydrogen sulphid, H_2S; *Arsenic*, As; *Antimony*, Sb; Cadmium, Cd; Tin, Sn.
2. Those precipitated *black* from an acid solution by hydrogen sulphid, H_2S; *Copper*, Cu; *Mercury*,

Hg; *Lead*, Pb; Silver, Ag; Bismuth, Bi; Platinum, Pt; Gold, Au.

3. Those precipitated by adding ammonia, NH_4HO, with ammonium sulphid, $(NH_4)_2S$; *zinc*, Zn; Nickel, Ni; Cobalt, Co; Iron, Fe; Chromium, Cr; Manganese, Mn; Aluminium, Al.
4. Those precipitated *white* by adding ammonia, NH_4HO, and sodium phosphate, Na_2HPO_4; the metals of the alkaline earths; *Barium*, Ba; Calcium, Ca; Strontium, Sr; Magnesium, Mg.
5. Those not precipitated by preceding reagents: the alkaline metals, Potassium, K, and Sodium, Na.

E. Neurotic Vegetable Alkaloids. *Morphin, Strychnin, Atropin.*

REAGENTS FOR TOXICOLOGY.

In addition to the reagents and apparatus mentioned on page 9 the following solutions are required for the experiments described in this division.

Acid, oxalic (normal solution).
Calcium hydrate (lime water).
Potassium iodid (1 in 20).
Potassium bichromate (1 in 10).
Ammonium chlorid (1 in 10).
Ammonium carb. (1 in 20).
Potassium ferrocyanid (1 in 10).
Ferrous sulphate (1 in 20).
Ferric chlorid (1 in 10).
Ammon. sulphhydrate.
Ferrous sulphid in lumps for making H_2S.

PRELIMINARY EXAMINATION FOR POISONS IN VOMITED OR OTHER SUSPECTED MATTERS.

(*a*) Note stains on the clothing or about the lips and mouth.

(*b*) Put vomited matters in a white dish and examine for odor, seeds, wing cases of cantharides, crystals, or powders.

(*c*) Take a small measured portion, say one-sixth or one-third, put in a clean jar, or test-tube, and stand in a bowl of warm water.

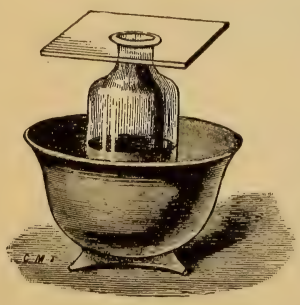

FIG. 29.

(*d*) *Test for Volatile Poisons.*—HCN by odor and by action of vapor on reagents applied to the under side of a glass cover to the jar (p. 119).

Phosphorus by odor and luminous vapor evolved in a dark room, by boiling a portion in a test-tube (p. 120).

(*e*) Test for *corrosive acids* by litmus paper, by examining stains on clothing, and by analyzing a sample of suspected substance for H_2SO_4, HNO_3, HCl.

(*f*) Test for *corrosive alkalies* by litmus paper or direct testing of suspected sample for KHO, NaHO, NH_4HO.

(*g*) Test for *metallic irritants.*

Apply Reinsch's test (p. 126) As, Sb, Hg; if there is a metal deposit on the copper, then make a sub-

limate in an open glass tube. Examine sublimate with lens, wash out with HCl, evaporate, and test residue with H_2S.

If there is no deposit on the copper, acidulate a portion with HCl and add H_2S. If it forms a black precipitate, test for Cu and Pb (pp. 130, 132).

(*h*) If the patient has narcotism or convulsions, apply Stas' method for *morphin* or *strychnin* by making a portion of the fluid vomit alkaline with sodium carbonate, which sets the alkaloid free. The alkaloid is then separated from the liquid which holds it suspended by agitating it with 4 volumes of ether or amylic alcohol. By letting the mixture stand, the ether floating will hold the alkaloid in separate solution, and may then be removed by a pipet. (Fig. 31.)

Fig. 30.

If the ether is allowed to evaporate on a saucer or watch-glass, the residue may be examined for strychnin (p. 136) and morphin (p. 137.).

If the indications point to morphin, then agitate a fresh portion of the original vomited fluid with amylic alcohol, as it is a better solvent for that alkaloid, separate, evaporate, and test (p. 137).

Note.—The methods indicated above are "rough and ready," and are such as might be applied quickly to vomited matter by a practitioner between his visits. They may serve to clear up a diagnosis before it is too late to help the patient. They would not answer for a juridical trial. The methods applied by expert analysts

are far more searching, and require much more apparatus, time, and special knowledge.

Before the analyst begins testing for the metallic irritants, he first gets rid of all organic matter by some such process as that of Fresenius. The finely divided substance is boiled with pure hydrochloric acid, while potassium chlorate is added from time to time until the solids are reduced to a straw-yellow fluid. This fluid is then treated with excess of sodium bisulphite and saturated with hydrogen sulphid until the metals are thrown down as sulphids. These sulphids are then treated with various tests for the metals.

CORROSIVE ACIDS AND ALKALIES.

The mineral acids and the alkalies corrode the tissues with which they come in contact and impart a strong corresponding reaction to the vomited matters. If alkaline antidotes have been administered, the reaction may be neutral, or even alkaline; and if alkaline poison has been treated by vinegar or lemon-juice the reaction may be changed to neutral or acid. The acids may have been converted into nitrates, chlorids, or sulphates. A complete examination of suspected material in the case of chlorids or sulphates requires an estimation of the *quantity* to determine if these salts are in excess. As nitrates are not found in the normal viscera or the ordinary gastric contents, their bare presence in notable amounts is very suspicious.

Besides determining the fact of acidity or alkalinity of the vomited matters, it may be necessary to determine the

degree. This is done by measuring the amount of a standard test solution necessary to neutralize the sample, using litmus or phenolphthalein as an indicator of the neutral point.

116. *Normal Solutions.*—The test solutions used in volumetric analysis are made chemically equivalent by adopting the standard of one gram of hydrogen dissolved in 1000 c.c. of water.

A *normal acid solution* contains in 1000 c.c. the amount of acid in grams representing one gram atom of replaceable hydrogen. For monobasic acids like HNO_3, the molecular weight in grams is dissolved in 1000 c.c. of water. For dibasic acids like H_2SO_4 and oxalic one atom of replaceable hydrogen requires only one-half the molecular weight in grams.

A *normal alkali solution* contains in 1000 c.c. the weight in grams of alkali neutralizing one gram-atom of acid hydrogen. For alkalies like KHO having but one hydroxyl group the whole molecular weight in grams is used. For those like $Ca(OH)_2$ having two hydroxyl groups one-half the molecular weight in grams is the proper amount.

Rules for Volumetric Analysis.—(1) Let some of the titrating solution run through the buret to wash it out; (2) fill the buret to the brim at first, then let it run out into the supply bottle until the upper surface stands at the 0 mark; (3) at the first titration let the flow be drop by drop, making frequent trials with the indicator; (4) to "read off" properly, bring the eye to a level with the top of the fluid and take the *lower* line of the zone made by the concave surface.

117. *Acidimetry Experiment.*—Measure one cubic centimeter of nitric acid, HNO_3, and fill to fifty cubic centimeters with water. Put this dilute acid into a capsule or beaker and add a few drops of litmus solution, or two drops of solution of phenolphthalein (1:100 alcohol). Fill a graduated buret (Fig. 5) to 0 with normal solution of potassium hydrate, KHO (56 gms. to 1000). Now let this alkaline solution run by drops into the capsule until the red litmus turns blue, or if phenolphthalein was used until the colorless fluid turns to persistent red. Note the number of cubic centimeters of KHO solution used, and calculate the absolute HNO_3 neutralized by multiplying the cubic centimeters of KHO used by 0.063.

$$HNO_3 + KHO = KNO_3 + H_2O$$
$$63 = 56 = \text{gms. in 1000 c.c.}$$

Potassium nitrate and water are formed.

Repeat this experiment with acids of different strengths.

If 1000 c.c. of the standard solution of KHO = 63 of HNO_3, then 1 c.c. = 0.063 of HNO_3 in acid liquid.

Operating on 1 gm. of the HNO_3, if 5 c.c. were required to neutralize, then $0.063 \times 5 = 0.315$ of absolute HNO_3 in each gram of acid liquid, or 31.5 per cent.

Operating on 5 gms. of the HNO_3, if 10 c.c. were required to neutralize, then $0.063 \times 10 = 0.63$ of HNO_3; in 5 gms. of acid liquid, $0.63 \times 20 = 12.6$ per cent.

If phenolphthalein is used as indicator, neutralization is denoted by a deep red color not further intensified by addition of more alkali.

118. *Alkalimetry.*—This procedure is like acidimetry, using blue litmus or red phenolphthalein as an indicator

and a normal solution of oxalic acid (63 gms. or one-half the gram molecular weight in 1000 c.c.) for testing. Each cubic centimeter = 0.056 gm. of KHO.

The acid is run in until the blue litmus turns red, or the red phenolphthalein becomes colorless. If the acid required to neutralize 1 c.c. of alkali numbers 8 c.c. then $8 \times 0.056 = 0.448$ gm. of KHO in 1 c.c. of solution.

NITRIC ACID, HNO_3. (*Aqua Fortis*).

Acidum nitricum, specific gravity 142 = 69.4 per cent. HNO_3.
Acidum nitricum dilutum (6 to 1) 10 per cent. HNO_3.

It causes death from acute laryngitis with edema or by perforation of the stomach or by secondary starvation. The tongue is stained yellow.

119. Test reaction with blue litmus paper.

120. Pour HNO_3 on copper slips in a test-tube and look for red-brown nitrous fumes, the liquid turning blue.

$$3Cu + 8HNO_3 = 3(Cu_2NO_3) + 4H_2O + N_2O_2.$$

Copper nitrate, water, and nitric oxid are formed, and nitric oxid in contact with air changes to red fumes of higher oxids.

121. Touch the skin and the clothing with a drop of HNO_3, and note in each case a yellow stain of picric acid, not removed by aqua ammonia.

122. HNO_3 turns a crystal of morphia yellow, but one of brucia red.

123. To dilute HNO_3 or a solution of a nitrate, add a green crystal of ferrous sulphate and a drop or two of

sulphuric acid. The crystal turns red-brown, ferric sulphate being formed.

ANTIDOTES.—Soapsuds, magnesia, chalk, or whiting suspended in milk, raw eggs. The stomach-pump must not be used, as the tube may perforate the softened tissues of the stomach.

HYDROCHLORIC ACID, HCl. *Spirit of Salt.*

Muriatic acid.
Acidum hydrochloricum, 31.9 per cent.
Acidum hydrochloricum dil. (3 to 13).

It is the natural acid of the gastric juice in the proportion of 0.25 per cent.

It causes death by corrosion, inflammation of the larynx perforation of the stomach, or secondary starvation.

124. Test with blue litmus paper.

125. Add HCl to a few grains of manganese dioxid, MnO_2, in a test-tube. Warm and note that chlorin gas forms and will bleach moist litmus paper held at the mouth of the tube.

$$MnO_2 + 4HCl = MnCl_2 + 2H_2O + Cl_2.$$

Manganese chlorid, water, and chlorin are formed.

126. Hold side by side the open mouths of bottles of HCl and of ammonia, NH_4HO, observing the white fumes of NH_4Cl formed.

127. Add to dilute HCl or a solution of sodium chlorid, NaCl, a drop of silver nitrate, $AgNO_3$. A white precipitate falls, soluble in ammonia but insoluble in HNO_3.

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$

Sodium nitrate and silver chlorid are formed.

Mercurous salts will yield with HCl a white precipitate of calomel, Hg_2Cl_2, insoluble in HNO_3 and blackened by NH_4OH.

Lead salts give a white precipitate of lead chlorid, $PbCl_2$, insoluble in NH_4OH.

128. Place upon a porcelain capsule one drop of dilute HCl and treat with a drop of a 0.5 per cent. alcoholic solution of di-methyl-amido-azo benzol. The acid will cause a cherry-red color. All free mineral acids will do this, but neutral or alkaline fluids are yellow. HCl (as little as 0.05 per cent.) will change Congo-red paper a decided blue, while lactic acid of 0.5 per cent. or less,—as it occurs in the gastric contents,—causes no material change.

129. *Uffelmann's* solution to distinguish lactic acid in gastric contents is made by adding a drop of neutral ferric chlorid solution to a mixture of 10 c.c. of a 4 per cent. solution of carbolic acid and 20 c.c. of water. The steel-blue liquid is turned yellow by lactic and butyric acids, but not affected by HCl when diluted to the strength of gastric juice (0.25 per cent.).

Reactions 128 and 129 are used clinically to determine the presence or absence of HCl in vomit. HCl is nearly always absent from gastric juice in cancer of the stomach. The value of these tests is impaired by interference from peptones, chlorids, and phosphates. (See Part II, Gastric Contents.)

Antidotes.—Soapsuds, magnesia in milk, raw eggs.

Nitromuriatic Acid. *Aqua Regia.*

Acidum nitrohydrochloricum, 4 of HNO_3 to 15 of HCl.
Acidum nitrohydrochloricum dil. (100 to 76 of H_2O).

An unstable mixture, owing to the HNO_3 yielding O to the HCl.

$$2HCl + 2HNO_3 = Cl_2 + N_2O_4 + 2H_2O.$$

It is detected by tests for HCl and for HNO_3. It dissolves gold foil. An exceedingly corrosive poison, with the same symptoms and antidotes as HNO_3.

Sulphuric Acid, H_2SO_4. *Oil of Vitriol.*

Nordhausen acid. $H_2S_2O_7$.
Acidum sulphuricum, specific gravity 184 = 96 per cent., H_2SO_4
Acidum sulph. dil., specific gravity = 10 per cent., H_2SO_4.
Acidum sulph. aromatic, 20 per cent., H_2SO_4, elixir vitriol.

A powerful corrosive poison, causing edema of larynx, perforation of stomach, and secondary starvation.

Antidotes.—Soapsuds, magnesia in milk, raw eggs. Burns on the lips and body treated with sodium bicarbonate and protectives.

130. Test with blue litmus paper.

131. Put a few drops of H_2SO_4 with a drop of water on a sheet of paper and warm gently until dry; a charred spot appears.

132. To dilute H_2SO_4, or a soluble sulphate, add barium chlorid, and a heavy white precipitate falls.

$$H_2SO_4 + BaCl_2 = 2HCl + BaSO_4.$$

Hydrochloric acid and barium suphate are formed.

133. A drop of H_2SO_4 applied to the black lining of the coat causes in time a moist red stain, which is removed by ammonia.

Strong H_2SO_4 when heated with Cu generates colorless suffocating fumes of SO_2

$$Cu + 2H_2SO_4 = CuSO_4 + SO_2 + 2H_2O.$$

Oxalic Acid, $H_2C_2O_4$. *Acid of Sugar.*

Potassium binoxalate, C_2HKO_4 (salt of sorrel or salt of lemon; ink-stain remover).

Oxalic acid and soluble oxalates when given in strong solution irritate the stomach, but rarely cause laryngitis or perforation. When absorbed, they cause death by nervous symptoms, collapse, heart failure, and stupor.

134. Oxalic acid added to lime-water gives a white precipitate of calcium oxalate.

135. Heat a crystal of oxalic acid in a test-tube. It is volatile and condenses in the cool part of the tube.

136. Precipitate a solution of oxalic acid with silver nitrate. It is white, and when separated by filtration and dried, it will disperse in vapor if heated on platinum foil.

137. Repeat the experiment with silver nitrate and divide the white mixture between two test-tubes. The precipitate of one will dissolve in nitric acid, that of the other in ammonia, whereas silver chlorid is insoluble in nitric acid.

Antidotes.—*Calcined magnesia* suspended in milk is the best. If this can not be had, then prepared chalk, whiting, plaster from the wall, or eggs. For the *mineral* acids soap or soapsuds may be used, but they are not antidotes to *oxalic* acid. The stomach-tube would probably injure the corroded tissues.

138. SHORT SYSTEM OF TESTS FOR MINERAL ACIDS.

Test for each acid with a fresh portion of the sample:

Add $BaCl_2$; white precipitate, insoluble in HNO_3, shows ..... H_2SO_4.

If Hg_2, Ag, or Pb are present, use $BaNO_3$ instead of $BaCl_2$.

Add $AgNO_3$.

1. White precipitate, soluble in NH_4HO and reprecipitated by HNO_3, shows..... HCl.

2. Yellowish precipitate, insoluble in NH_4HO, shows..... HI.

HCl may also be present. Filter; to filtrate add HNO_3; precipitate shows ..... HCl.

Add strong H_2SO_4, then strong, fresh solution of $FeSO_4$, in such a way that the two fluids do not mix; shake them very gently; a brown color at junction shows ..... HNO_3.

Add HCl.

1. Effervescence and smell of H_2S shows.... H_2S.

2. Effervescence and no smell shows..... H_2CO_3.

CAUSTIC AND CARBONATED ALKALIES.

Potassium Hydroxid, KHO. *Caustic Potash.*

Liquor potassii hydroxid, KHO.

Potassii carbonas, K_2CO_3.

Caustic potash is a deep corrosive of great energy; causes a soapy taste, pain in mouth, throat, and stomach, vomiting and purging. Death may ensue from perfora-

tion of the stomach or from starvation due to closure of the esophagus.

139. Test with red litmus or turmeric paper.

140. Dilute the KHO with an equal part of water, and add HCl until the reaction is neutral to litmus paper.

$$KHO + HCl = H_2O + KCl.$$

Water and potassium chlorid are formed.

141. To this potassium chlorid add platinum chlorid; a yellow granular precipitate falls slowly. Ammonium salts give a similar result.

$$2KCl + PtCl_4 = PtCl_4.2KCl.$$

A double salt, potassioplatinic chlorid, is formed.

142. Heat platinum foil or wire in a Bunsen's burner until the flame is colorless; then touch it with moist KHO and heat again; a violet tint appears. If mixed with yellow, observe through deep blue glass, which shuts out the yellow rays and leaves the violet.

ANTIDOTES.—Weak vinegar, lemon-juice, milk, and oils (castor or olive).

SODIUM HYDROXID, NaHO. *Caustic Soda.*

Liquor sodii hydroxidi NaHO.

Concentrated lye.

Sodii carbonas. *Sal soda, washing soda,* corrosive in all respects like potash.

143. Test for alkaline reaction.

144. Add HCl to neutralize.

145. Heat NaHO on platinum foil; the flame is colored yellow.

This test is so delicate as to reveal a trace of sodium present almost everywhere.

ANTIDOTES.—Weak vinegar, lemon-juice, milk, and oils.

AMMONIUM HYDROXID, NH_4HO. *Ammonia Water.*

Aqua ammonia fortior, 28 per cent. by weight.
Aqua ammonia, 10 per cent., *hartshorn.*
Spiritus ammoniæ.

It causes suffocation, with inflammation of mouth, larynx, esophagus, and stomach.

146. Note the odor of aqua ammonia or ammonium carbonate.

147. Test the fumes with moist red litmus paper; they turn it blue.

148. Neutralize with HCl; observe the white fumes of ammonium chlorid, NH_4Cl, and note that the ammoniacal odor ceases.

149. Boil the last product, NH_4Cl, with KHO; the odor of free ammonia may be perceived.

$$NH_4Cl + KHO = KCl + NH_4HO.$$

Potassium chlorid and ammonia are formed.

ANTIDOTES TO THE ALKALIES.—*Vinegar and water* (dilute acetic acid), lemon-juice, oils, butter, milk. Do not use the stomach-pump.

VOLATILE POISONS.

Under this head are included carbolic acid, hydrocyanic acid, and phosphorus as the most important, and chloroform, chloral, ether, alcohol, nitrobenzene, besides some others of less importance in toxicology. Peculiar odors are distinctive of nearly all the volatile poisons.

Acid Carbolic, C_6H_5HO. *Phenol. Phenyl Hydrate.* It is an escharotic and cardiac depressant, causing pain in the stomach, coma, collapse.

150. Note the odor.

151. A drop applied to blue litmus paper leaves a greasy stain, but does not redden the paper.

152. To a dilute solution of carbolic acid add a few drops of nitric acid and warm gently as long as red fumes are given off, then neutralize with potassium hydroxid and a yellow crystalline precipitate forms of picrate of potassium.

153. Strong bromin water causes a whitish-yellow precipitate.

154. To a little HCl add two drops of carbolic acid, mix and add one drop of HNO_3; a purple-red color appears.

155. To a weak solution of carbolic acid add a few drops of $FeCl_3$; a permanent violet-blue color results. Creasote yields to the $FeCl_3$ a green or brown color.

Antidotes.—*Sodium sulphate, saccharate of lime,* lime-water, magnesia, eggs. Alcohol or whisky promptly evacuated. The stomach-pump may be used freely. Treat symptoms by stimulants.

Acid Hydrocyanic, HCN. *Prussic Acid.*

Acidum hydrocyanicum dil., 2 per cent HCN.
Potassium cyanidum, KCN.
Oil of bitter almonds has 5 to 14 per cent. HCN.
Laurel water, peach, cherry, and plum kernels.

The symptoms produced by it are convulsions, coma, and collapse. It is not a corrosive poison, but a syncopant or hyposthenisant.

156. Note the odor of bruised peach kernels.

157. Put HCN or potassium cyanid solution KCN, into a test-tube, and add a few drops of sulphuric acid. Cover with a watch-crystal or saucer wet with one drop of silver nitrate and warm gently. A white spot appears, which is soluble in warm nitric acid.

$$KCN + AgNO_3 = KNO_3 + AgCN.$$

Potassium nitrate and silver cyanid are formed.

158. Cover with another watch-crystal wet with potassium hydrate. After a few minutes touch with ferrous sulphate and with ferric chlorid, and finally with one drop of HCl. A precipitate of Prussian blue (ferrocyanid of iron) forms.

159 Cover with another watch-crystal or saucer wet with ammonium sulphid.

$$HCN + NH_4HS = 2H + NH_4SCN.$$

White ammonium sulphocyanid is formed.

Now touch this with ferric chlorid, $FeCl_3$, and it turns to blood-red sulphocyanid of iron.

$$3NH_4SCN + FeCl_3 = 3NH_4Cl + Fe(SCN)_3.$$

ANTIDOTES.—Affusions of cold water, ammonia, chlorin and a mixture of ferrous sulphate, ferric chlorid and magnesia, wash out the stomach with water containing hydrogen peroxid or potassium permanganate alternately.

PHOSPHORUS, P.

Oleum phosphoratum, 1 per cent. of P.
Heads of matches.
Rat paste contains 5 per cent. of P.

Its poisonous effects upon the stomach are those of a local irritant. When absorbed, it causes general fatty degeneration. The liver and heart are markedly affected, causing jaundice and syncope.

160. Boil a match-head in water with a few drops of some acid, and in a dark closet note the luminous appearance.

161. Observe that it has an odor like garlic.

162. Clasp over the mouth of the tube filter paper moist with silver nitrate. The phosphorous vapor will cause a dark stain of phosphid of silver.

ANTIDOTES.—Crude turpentine, magnesia in milk.

Antal's antidote is potassium permanganate in weak solution given freely. Copper sulphate is of doubtful value. Hydrogen peroxid diluted alternating with dilute potassium permanganate may be used to wash out the stomach.

CHLOROFORM, $CHCl_3$. *Trichlormethane.*

It is an anesthetic and causes death by failure of respiration and heart's action.

Note the odor and taste. Shake it with water; it separates as a bottom layer.

163. A strip of paper wet with chloroform and burned in the Bunsen flame imparts a green tint and yields acid fumes which redden moist blue litmus paper. If the glass rod wet with ammonia is held above the flame, white clouds of ammonium chlorid show that HCl is a product of combustion.

164. To an alcoholic solution of potassium hydrate add a few drops of anilin, $C_6H_5.NH_2$, and one of chloroform.

Immediately, or perhaps after gentle heat, a penetrating, disagreeable, and peculiar odor is emitted.

$$CHCl_3 + 3KHO + C_6H_5.NH_2 = C_6H_5.NC + 3KCl + 3H_2O.$$

The offensive benzo-isonitril and potassium chlorid and water are formed.

165. Warm a solution of beta-naphthol in KHO, add chloroform and a blue color is formed.

166. Dissolve about a grain of resorcin in 1 c.c. of chloroform, add 5 drops of NaHO and boil, a red color develops.

ANTIDOTES.—Treat symptoms by lowering the head, drawing out the tongue, cold affusion, artificial respiration with oxygen, electricity.

CHLORAL HYDRATE, C_2HCl_3O. *Trichloraldehyd.*

A narcotic poison causing coma and syncope.

167. Dissolve a small piece of chloral hydrate in water add potassium hydrate, and boil.

$$C_2HCl_3O + KHO = CHCl_3 + KCHO_2.$$

Chloroform and potassium formate are formed.

The chloroform vapor may be recognized by its odor or recovered by distillation, and the tests for chloroform applied to the distillate.

168. Apply the anilin test (§ 164) to a portion of the mixture in the test-tube, and benzo-isonitril may be detected by its offensive odor.

169. Remove the chloroform from the above mixture (§ 168) by boiling, then add ammonia and a crystal of silver nitrate. The silver is reduced by potassium formate with formation of a metallic mirror on the tube.

Emetics, such as apomorphina, 1/16 grain hypodermically.

ANTIDOTES.—Wash out the stomach with tea and coffee, and treat symptoms with cold affusions, sinapisms to epigastrium, artificial respiration, and electricity.

ALCOHOL, $C_2H_5.HO$.

Present in malt, vinous, and spirituous liquors. It is an inebriant.

170. To solution of potassium bichromate, $K_2Cr_2O_7$, add a few drops of sulphuric acid, and dilute with water. If alcohol is now added and the admixture gently heated, it will turn green, and aldehyd vapor will be recognized by its odor.

$$3C_2H_5OH + K_2Cr_2O_7 + 4H_2SO_4 = 3C_2H_4O + K_2SO_4.Cr_2(SO_4)_3 + 7H_2O.$$

Aldehyd and green potassiochromic alum are formed.

171. Dissolve a crystal of iodin in some alcohol. Add potassium hydrate until the brown color disappears, and after gentle heat a yellow precipitate of iodoform falls.

$$C_2H_5OH + 6KHO + I_8 = CHI_3 + CHO_2K + 5KI + 5H_2O.$$

Iodoform, potassium formate, potassium iodid, and water are formed.

ANTIDOTES.—Emetics or stomach-pump, cold affusions, ammonium carbonate.

METALLIC IRRITANTS.

ARSENUM, As. *Elementary Arsenic.*

Fly-paper contains As in a paste.

Hydrogen arsenid, arsenia (AsH_3), is the most poisonous form and must not be inhaled.

Arsenic trioxid, As_2O_3, *acidum arsenosum*, white arsenic

ratsbane. It is usually called arsenic, and is the most commonly used poison for homicidal purposes.

"Aqua Toffana," a boiled solution of As_2O_3.

"Rough on Rats" contains As and As_2O_3.

"Buffalo Carpet Moth Annihilator" contains As_2O_3.

Liquor acidi arsenosi, one per cent. As_2O_3 with HCl.

Liquor potass. arsenitis (Fowler's) one per cent. As_2O_3 with $KHCO_3$.

Liquor sodii arsenitis (Harle's), one per cent. As_2O_3 with Na_2CO_3.

Liquor sodii arsenatis (Pearson's), one per cent. Na_2HAsO_4.

Liquor arseni et hydrarg. iodid. (Donovan's), one per cent. AsI_3.

Arsenic pentoxid, As_2O_5, *acidum arsenicum*, H_3AsO_4.

Realgar, As_2S_2.

Orpiment, As_2S_3, *King's yellow.*

London purple, *arsenical dye refuse.*

Paris green, *Schweinfurth green*, impure acetoarsenite copper, $Cu(C_2H_3O_2)_2+3(CuAs_2O_4)$, a very common and dangerous poison.

Scheele's green, *copper arsenite and hydroxid.*

Cacodylic Acid, $As(CH_3)_2OOH$, comparatively innocent though it contains the equivalent of 72 per cent. of As_2O_3. In overdoses it is poisonous.

Sodium Cacodylate, $As(CH_3)_2O_2Na$, poisonous in overdoses.

"Atoxyl," *sodium aminophenyl arsenate*, $C_6H_4(NH_2)$. (AsO.OH.ONa) is well borne in large doses but it may prove poisonous in excess. Three grains contain 3/4 gr.As.

The arsenical compounds are irritant poisons, inflaming living tissue by contact, usually in about half an hour, causing symptoms of inflammation of stomach and bowels, such as epigastric pain, vomiting, thirst, and purging. Sometimes they cause profound shock and death by coma. Slow or chronic poisoning induces cachexia, diarrhea, paralysis, and death by wasting.

In the Solid Form.

172. Put into a reduction tube a small quantity of As_2O_3 mixed with six times its bulk of dried flux (made of dry sodium carb., 3 parts, to potass. cyanid, 1 part).

FIG. 31

FIG. 32.

FIG. 33.

Heat the mixture and observe the formation of a dark mirror ring of As on the tube. (*b*. Fig. 31.) Break off the closed end of the tube and heat the ring. A current of air plays through the tube and oxidizes the As to As_2O_3,

which is deposited in the cool part of the tube as a white ring of minute octahedral crystals. (Figs. 32 and 33.)

In the Liquid Form.

173. To a solution of As_2O_3 add a few drops of HCl and pass through it hydrogen sulphid, H_2S; a lemon-yellow precipitate falls, soluble in ammonia and in NH_4HS, but not in HCl.

$$2H_3AsO_3 + 3H_2S = As_2S_3 + 6H_2O.$$

Arsenic trisulphid and water are formed.

174. To a neutral, alkaline, or feebly acid solution of As_2O_3 add silver ammonionitrate; it throws down a canary-colored precipitate of silver arsenite, Ag_3AsO_3.

175. To a solution of As_2O_3 add copper ammonio-sulphate; it throws down a green precipitate of copper arsenite, $CuHAsO_3$.

176. *Fleitmann's Test.*—Into a test-tube put some strong potassium hydrate and a few pieces of pure zinc or aluminium; clasp over the mouth of the tube paper wet with silver nitrate, and boil. If the paper is not stained, the materials used for this test are free from arsenic.

$$Zn + 2KHO = H_2 + K_2ZnO_2.$$

Hydrogen and potassium zincate are formed.

Now add a few drops of arsenical solution to the same mixture, and boil as before; a black stain appears on the paper.

$$AsH_3 + 6AgNO_3 + 3H_2O = 6Ag + H_3AsO_3 + 6HNO_3.$$

Black metallic silver, arsenous acid, and nitric acid are formed.

177. *Reinsch's Test.*—Into a solution of As_2O_3, in a

test-tube, put a few drops of HCl and a clean slip of copper; then boil for a few minutes. When the slip has a steel-gray coat of As, remove it; dry with blotting paper, and heat in an open glass tube. Note the white ring of As_2O_3 crystals.

178. *Marsh's Test.*—Into a hydrogen-generating apparatus (Fig. 34) put some pure zinc and add dilute sulphuric acid (H_2SO_4 part one to H_2O parts four). *Wait* fifteen minutes, until the explosive mixture of air and hydrogen has escaped; then light the H gas at the jet and hold in the flame a piece of cold porcelain. If no stain is made, then arsenic and antimony are absent from the materials. Now add solution of arsenous acid, and try the flame again with porcelain. A brownish-black metallic mirror of As appears.

FIG. 34.

$$3Zn + 3H_2SO_4 + H_3AsO_3 = 3ZnSO_4 + 3H_2O + AsH_3.$$

Zinc sulphate, water, and hydrogen arsenid are formed in the generator.

When the AsH_3 is burned, it is decomposed, forming $H_2O + As$. The arsenic, when deposited on the cold porcelain, is recognized by being quickly dissolved when treated with liq. sodæ chlorinatæ or a solution of calx chlorinata. If treated with nitric acid and gently evaporated, it is oxidized to arsenous acid as a whitish deposit. The arsenious acid touched with a drop of strong solu-

tion of $AgNO_3$ gives red silver arsenate. The original As deposit on porcelain will dissolve slowly in ammonium sulphid, leaving a yellow residue on evaporation. This residue is soluble in NH_4HO, but insoluble in HCl.

179. Use a fresh hydrogen apparatus with solution of an antimony salt,—say tartar emetic,—repeating the same procedure. On the porcelain a sooty stain appears which is not removed on adding a drop of liq. sodæ chloratæ. It is soluble in nitric acid, but does not afterward turn red with silver nitrate. It is freely soluble in ammonium sulphid with an orange-red residue insoluble in NH_4HO.

180. *Arsenical Wallpaper.*—Take 4 square inches of suspected paper, cut up finely, moisten with strong HCl, heat gently on sand bath. Extract with water brought to a boil, filter and test the filtrate with Reinsch's or Marsh's test.

181. Make the *antidote—ferric hydroxid*—by adding to tinctura ferri chloridi some calcined magnesia in excess, and shake well together. If three fluidounces of tinct. ferri chlor. are mixed with three drams of magnesia suspended in water, the mixture will be an antidote for ten grains of arsenious acid.

$$Fe(HO)_3 + H_3AsO_3 = FeAsO_3 + 3H_2O.$$

182. To a solution of ferric chlorid add enough ammonia water to precipitate brown ferric hydroxid Throw the brown mass upon a straining cloth or filter and wash out the excess of ammonia under the tap of water. Mix and stir the brown precipitate with a solution of arsenic in a beaker and after five minutes filter and test

with Reinsch's test. Arsenic will be absent from the solution.

Antidotes to Arsenic.—Milk, eggs, hydrate magnesia, dialysed iron, *moist ferric hydroxid*, ferri hydroxidum cum magnesii oxido.

Antimony, Sb. Tartar Emetic, $K(SbO)C_4H_4O_6$

Antimony oxid, Sb_2O_3.
Antim. et potass. tartras, *tartar emetic.*
Vinum antimonii, 0.4 per cent. of tartar emetic.
Syr. scillæ comp. contains tartar emetic.

Tartar emetic has a faint metallic taste, and acts as an irritant poison, with symptoms like those produced by arsenic.

183. Pass hydrogen sulphid into a solution of tartar emetic, acidified with dilute HCl or tartaric acid. An orange-red precipitate of antimony sulphid forms. Divide this precipitate in three portions; one is insoluble in aq. ammonia, one is soluble in ammonium sulphid, and the third is soluble in hydrochloric acid. Arsenic sulphid is insoluble in HCl.

184. Repeat Reinsch's test (§ 177), using solution of tartar emetic instead of arsenic. The deposit on copper is bluish, and when heated in an open glass tube yields a white sublimate, generally amorphous, though it may be octahedral. If this sublimate is dissolved in HCl and evaporated, the residue turns orange with H_2S.

185. *Repeat* Marsh's test (§ 178), using solution of tartar emetic instead of arsenic. The stain on porcelain is sooty, barely soluble in liq. sodæ chlorinatæ but soluble in NH_4HS, leaving an orange-red residue.

186. Put a drop of solution of tartar emetic on platinum foil, add a drop of HCl, and touch the platinum through the drop with a slip of zinc. A black spot of antimony appears, which turns orange-yellow with ammonium sulphid. The orange spot is insoluble in ammonia but soluble in HCl.

ANTIDOTES TO ANTIMONY.—*Acid tannic*, tea, vegetable astringents, dialysed iron, moist ferric hydroxid, stomach-pump.

COPPER, Cu.

Copper sulphate, $CuSO_4$, *blue vitriol.*
Copper subacetate *verdigris.*

The salts of copper are irritants in full doses. If impure, they are apt to contain traces of arsenic.

187. Treat acidulated solution of copper sulphate, $CuSO_4$, with hydrogen sulphid, and a brown-black precipitate falls.

188. Dip into solution of $CuSO_4$ a bright steel needle; it soon has a red coat of metallic copper.

189. Treat a weak solution of $CuSO_4$ with potassium ferrocyanid; a brown precipitate falls.

190. To a solution of $CuSO_4$ add a drop of ammonia; a bluish precipitate falls, but on addition of more ammonia this clears into a deep blue solution.

$$CuSO_4 + 2NH_4HO = CuSO_4.2NH_3 + 2H_2O.$$

Blue ammoniosulphate of copper and water are formed.

ANTIDOTES.—Warm milk, raw eggs, sodium bicarb. followed by emetic, pure ferrocyanid of potassium.

Mercury, Hg. Corrosive Sublimate, $HgCl_2$.

Mercuric chlorid, $HgCl_2$, *bichlorid.*
Mercurous chlorid, HgCl, *calomel.*
Mercuric oxid, HgO, *red and yellow.*
Mercurous oxid, Hg_2O, *black.*
Mercuric iodid, HgI_2, *red.*
Mercurous iodid, HgI, *green.*

The symptoms caused by poisonous doses of the mercuric salts are those of active irritants—*i.e.*, pain, vomiting, thirst, purging, depression.

Corrosive sublimate is more prompt in its effects than arsenic, having a strong taste and corroding the tissues at once.

Chronic poisoning is characterized by salivation, tremors, wasting, debility.

191. Treat $HgCl_2$ with KI; it forms a yellow and red precipitate, which finally clears up in excess of KI.

$$HgCl_2 + 2KI = HgI_2 + 2KCl.$$

192. Treat calomel, HgCl, with lime-water; it turns to black oxid, Hg_2O.

$$2HgCl + Ca2HO = Hg_2O + CaCl_2 + H_2O.$$

Black mercurous oxid, calcium chlorid, and water are formed.

193. Treat corrosive sublimate, $HgCl_2$, with lime-water; it turns to yellow oxid, HgO.

194. Perform Reinsch's test (§ 177) with corrosive sublimate solution without boiling; the copper takes a gray coat of metal, Hg.

Dry the copper and heat it in an open tube; a subli-

mate of minute gray globules forms. Heat this sublimate with iodin; it forms red mercuric iodid, HgI_2.

Galvanic Test.—On a gold ring or coin put a drop of solution of corrosive sublimate; with a steel needle or knife touch the gold through the drop, and a silver-like spot of metal, Hg, appears. This stain is removable by gentle heat over a flame.

ANTIDOTES.—Raw eggs, flour in milk, emetics.

LEAD, Pb.

Plumbi oxidum, PbO, *litharge.*
Plumbi oxid, rubrum, Pb_3O_4, *red lead.*
Plumbic oxychlorid, $Pb_4O_3Cl_2$, *Turner's yellow.*
Plumbi acetas, *sugar of lead.*
Liq. plumbi subacetatis, *Goulard's extract.*
Plumbi chromas, $PbCrO_4$, *chrome yellow.*
Plumbi carbonas, $(PbCO_3)_2Pb(HO)_2$, *white lead.*

All the salts of lead are poisonous except, perhaps, the sulphate and the sulphid. The carbonate is the most active.

Lead-poisoning is characterized by obstinate colic and constipation, with a blue line on the margin of the gums. Later on, palsy and wasting of the extensor muscles of the forearm, cachexia, joint pains, mania, or convulsions appear.

195. Put a crystal of lead acetate on a piece of charcoal and heat with the tip of a blowpipe flame. A globule of metallic lead soon forms with a yellow margin.

196. To a solution of lead salt add hydrogen sulphid; a black precipitate of lead sulphid, PbS, falls; insoluble in KHO and NH_4HS but soluble in boiling dilute HNO_3.

197. To another portion of lead solution add acid sulphuric; a white precipitate of lead sulphate, $PbSO_4$, falls.

198. To another portion add potassium iodid; a yellow precipitate of lead iodid, PbI_2, falls.

199. To another portion add K_2CrO_4; a yellow precipitate of lead chromate appears.

ANTIDOTES.—Magnesium sulphate and other soluble sulphates with milk and opium for colic, potassium iodid and iron for chronic poisoning.

$$PbCO_3 + MgSO_4 = PbSO_4 + MgCO_3.$$

SILVER, Ag.

Argenti nitras, $AgNO_3$. *Lunar caustic* is an irritant poison. Prolonged administration causes a slaty-blue color of skin.

200. To a solution of silver nitrate add H_2S; it precipitates black silver sulphid, Ag_2S, insoluble in dilute acids, alkalies, alkaline sulphids, but soluble in boiling HNO_3.

201. To another portion cautiously add NH_4HO; it gives a brown precipitate of Ag_2O, which readily dissolves in excess of NH_4HO.

202. HCl gives white curds of AgCl, soluble in NH_4HO.

203. KI gives yellow precipitate of AgI, insoluble in NH_4HO.

204. H_2SO_4 yields no precipitate whatever.

ANTIDOTES.—Common salt, raw eggs, and milk.

Zinc, Zn.

Zinci oxidum, ZnO, *zinc white.*

Liq. zinci chloridum, $ZnCl_2$, *Burnett's disinfectant*, also used in a soldering fluid, and as preservation injection for bodies in the dissecting room.

Zinci sulphas, $ZnSO_4 + 7H_2O$, *white vitriol.* The salts of zinc are irritant poisons, inducing secondary depression of the nervous system.

205. To solution of a zinc salt add ammonium hydrate. A white precipitate of zinc hydrate, $Zn(OH)_2$, falls, soluble in excess of the ammonium hydrate.

206. To another portion add first ammonium chlorid and then ammonium hydrate. There is no precipitate. Now add hydrogen sulphid. White zinc sulphid is thrown down.

207. To a neutral or alkaline solution of zinc salt add ammonium sulphid. A white precipitate, ZnS, is thrown down.

Antidotes.—Raw eggs, milk, tea, vegetable astringents.

Barium, Ba.

The chlorid and nitrate are irritant poisons.

208. From solution of a barium salt, potassium hydrate will throw out white barium hydrate, $Ba(OH)_2$. Ammonium hydrate has no such effect when added to another portion.

209. To the clear solution with ammonia add sodium phosphate, Na_2HPO_4; it forms a white precipitate of barium phosphate, $BaHPO_4$.

211. Analysis of a Neutral or Slightly Acid Solution of Ordinary Salts of One of the Common Metals.

Add hydrochloric acid.

Precipitate Hg (ous), Pb, Ag. Collect, wash, and add NH_4HO. Hg ppt., blackened Pb ppt., still white. Ag ppt., dissolved. Sb and Bi may also be precipitated by HCl, but are dissolved on adding more HCl.

If HCl gave no precipitate, the metal is still in the liquid; pass H_2S through the solution.

Precipitate Cd, Cu, Hg(ic), Pb, Bi, As, Sb, Sn, Au, Pt. Collect, wash, add NH_4HS.

Insoluble.		Soluble.	
Cd, yellow.		As (ous & ic)	yellow.
Cu	black.	Sn (ic)	yellow.
Hg(ic)	black.	Sb, orange,	
Pb	black.	Sn (ous)	black.
Bi	black.	Au	black.
		Pt	black.

Apply special tests for each to the original solution. For these, see the previous pages.

If H_2S gave no precipitate, add NH_4Cl, NH_4HO and NH_4HS.

Precipitate. Zn, Mn, Co, Ni, Al, Fe, Cr.

Zn, Al — white.
Cr, green.
Mn, skin-tint.
Ni, Co, Fe — black.

Test specially for each in original solution. See previous pages.

If NH_4HS gave no precipitate, add $(NH_4)_2CO_3$.

Precipitate Ba, Sr. Ca, Collect, wash, dissolve in $HC_2H_3O_2$ add K_2CrO_4.

Ppt.	Sol.
Ba.	Sr, Ca. Add dil. H_2SO_4.
	Ppt. Sr—Ca.

If $(NH_4)_2CO_3$ gave no precipitate add $(NH_4)_2HAsO_4$.

Ppt.	
Mg.	If no precipitate, test original solution in flame on loop of Pt wire. Li, crimson. Na, yellow. K, violet. If neither, test orig. sol. for NH_4.

210. To a solution of barium salt add acid sulphuric or any soluble sulphate. A white precipitate, $BaSO_4$, falls.

ANTIDOTES.—Magnesium sulphate or any soluble sulphate, eggs, and milk.

NEUROTIC ALKALOIDS.

STRYCHNINA, $C_{21}H_{22}N_2O_2$. *Alkaloid from Strychnos Nux Vomica.*

Strychnina sulphate and nitrate.
Tincture and extract of nux vomica.
"Battle's Vermin Killer" has 23 per cent.
"Butler's Vermin Killer" has 5 per cent.
"Gibson's Vermin Killer" has ½ gr. in each.

All the above preparations have a very bitter taste, and in poisonous doses cause spinal symptoms, such as convulsions, epigastric pain, and death by exhaustion or prolonged spasm of respiratory muscles, the brain remaining clear.

212. Note the intensely bitter taste of one drop of the solution.

213. On a white dish put a crystal of strychnin, and add to it a drop of acid sulphuric. It dissolves without change of color. Near by upon the same dish put a crystal of potassium bichromate and let the acid solution flow over it. At the line of contact a transient play of colors will be seen in the following order: blue, violet, red, and yellow.

214. Nitric acid makes with it a colorless solution. If brucin is present as an adulteration, a red color is produced.

215. Insert a few drops of strychnin solution under the skin of a frog's back. Within a few minutes it will be convulsed and die tetanized. A number of agents will cause spasms in the frog when inserted hypodermically. A more discriminating method is to immerse a fresh young frog in the suspected fluid. In the course of an hour absorption of strychnin will induce the characteristic spasms.

ANTIDOTES.—Emetics (such as apomorphina, 1/16 gr. hypodermically), potassium permanganate 4 grains in 11 fluidounces of water, drafts of tea or other vegetable astringents, chloroform by inhalation, chloral, and potassium bromid by enema.

MORPHINA, $C_{17}H_{19}NO_3$. *Alkaloid from Opium Poppy.*

Morphina acetate, hydrochlorate, and sulphate.

Opium and all its preparations contain it. It is present in the proprietary opiates sold to relieve pain and induce sleep, as *Godfrey's*, *Dalby's*, *Mrs. Winslow's*, *Battle's*, *Nepenthe*, *Chlorodyne*, and various "opium cures."

It is a narcotic poison, causing stupor with contracted pupils and slow respiration, deepening into coma and death by arrest of respiration.

216. On a white dish put a crystal of morphin sulphate, and touch it with a drop of nitric acid; it turns at first orange-red and fades to yellow.

217. To a neutral solution or a crystal of morphin sulphate add pure neutral ferric chlorid, $FeCl_3$; a deep blue color appears.

218. To morphin solution add iodic acid in solution; a yellow color of reduced iodin appears. If the yellow

liquid is shaken with chloroform, the iodin separates in a violet-colored layer.

ANTIDOTES.—Emetics (such as apomorphina, 1/16 gr. hypodermically), washing out the stomach with strong tea, electricity, flogging, artificial respiration, brandy, potassium permanganate freely in weak solution (four grains to four ounces of water), hypodermic injection of cocain, strychnin or atropia.

OPIUM AND ITS CHARACTERISTIC MECONIC ACID.

219. To a portion of solution of meconic acid or a decolorized infusion of tincture of opium add ferric chlorid, $FeCl_3$ (pure and neutral); a brown-red color appears. This color persists even if treated with mercury bichlorid, $HgCl_2$, or after boiling with dilute HCl.

ATROPINA, $C_{17}H_{23}NO_3$. *Alkaloid of Atropa Belladonna.*

Daturina, an alkaloid of datura stramonium.
Hyoscyamina, an alkaloid of hyoscyamus niger.
Duboisina, an alkaloid of duboisia myoporoides.

All of the above are practically identical. They are active deliriant poisons, causing dilatation of the pupils, flushing of the face, dryness of the throat, muscular weakness, dizziness, delirium, and coma.

220. A small portion of atropin, if heated with strong acid sulphuric, does not change color, but gives off an odor of roses.

221. Into a test-tube put a few drops of acid sulphuric and a crystal of potassium bichromate, and heat them. If a fraction of a grain of atropin is now dropped in, a green color and an aromatic odor may be perceived.

ANTIDOTES.—Emetics, washing out the stomach with strong tea or other preparation containing tannin, potassium permanganate 4 grains in 4 fluidounces of water.

222. ANALYTIC TABLE FOR COMMON ALKALOIDS.—(*Senier's.*)

PART I.

Morphin, Brucin.		Strychnin, Salicin.*	
To a small quantity on a white plate add strong nitric acid.		If no morphin or brucin, moisten a small quantity on a white plate with strong sulphuric acid.	
An orange color, decolorized by Sn, Cl_2, $Na_2S_2O_3$, or NaHS = *morphin.*† Confirm by $FeCl_3$ (neut.); which gives with morphin or its salts a blue color. HIO_3 is decomposed by morphin or its compounds, with liberation of iodin, which may be recognized by starch.	*A red color.* To a few grains in a test-tube add a drop or two of HNO_3, warm to boiling and dilute with water; then add a few drops of stannous chlorid. A violet color = *brucin.*	*A deep red color = salicin.* Confirm by boiling the substance with water to which has been added a few drops of dilute H_2SO_4. Then make solution alkaline and examine for glucose.	*No color, or only a slight color.* Draw a moistened crystal of $K_2Cr_2O_7$ across the acid film, when a transient play of colors, violet to red = *strychnin.*

*Salicin is a glucosid with some reactions like those of the poisonous alkaloids.

†Strychnin of commerce often gives an orange or red color, due to contamination with brucin.

223. Analytic Table for Common Alkaloids.

Part II.

Quinin, Quinidin. Cinchonin, Cinchonidin.

If morphin, brucin, salicin and strychnin are absent, make a saturated solution in hot water, and cool down to about 60° F., removing the crystals which have deposited by filtration. If the substance is an alkaloid or an acid salt, it must be neutralized. To one volume of this solution in a test-tube add exactly half a volume of ether and then ammonia solution in slight excess. Agitate, and set aside for a quarter of an hour.

Precipitate entirely soluble in the ether.	*A crystalline layer between the ethereal and aqueous solutions.*	*Precipitate insoluble in ether = cinchonin.*
To a portion of the saturated aqueous solution add rather more than an equal bulk of ammonia solution (sp. gr. 0.96) and agitate. The precipitate dissolves completely = *ordinary sulphate of quinin.* If precipitate does not dissolve completely, add to another portion of the aqueous solution $(NH_4)_2C_2O_4$ and agitate (no precipitate at once with ordinary sulphate of quinin). A bulky crystalline precipitate = *quinin.*	To a portion of the aqueous solution add sodium tartrate and agitate. A bulky crystalline precipitate = *cinchonidin.* If no precipitate, or only a trace, add to another portion of the aqueous solution KI. A heavy white precipitate = *quinidin.* If the precipitate be semi-liquid, it contains cinchonidin or other alkaloids, the hydriodates of which are soluble in weak spirit.	Confirm by other reactions. Chlorin and ammonia give a yellow precipitate.

PART IV.

STUDY OF NORMAL MILK

AND

MILK EXAMINATION.

COMPOSITION OF NORMAL MILK.

Milk is a sweet, opaque, bluish-white fluid, with a peculiar odor, holding in solution casein, albumin, sugar, and mineral salts. Its turbidity is due to the minute fat or butter globules which are suspended in it.

FIG. 35.

Percentage Composition of Normal Milk.	Cow.	Human.
Water	85.70	88.90
Solids, as tabulated below	14.30	11.10
Casein	4.82	3.90
Albumin and globulin	0.58	
Butter or fat	3–4.30	2.65–4.00
Milk sugar	4.00	4.35
Salts	0.50	0.20

The composition is somewhat variable, the proportion of casein rising when the breasts are emptied frequently, and the last portions obtained containing more butter.

Microscopically, the milk is found to be composed of minute brilliant *oil globules* in thin envelopes of casein,

suspended in the clear milk plasma. Immediately after delivery of the young, the milk is relatively poor in casein but rich in fatty matter, which exists to a considerable extent in the form of *colostrum* masses. (Fig. 35.)

MORBID MILK.

The constitution and the food value of human milk are impaired when the mother is not healthy. It is affected injuriously by undue emotional excitement. It will contain an appreciable amount of certain medicines given to the mother, and thus the infant be affected secondarily.

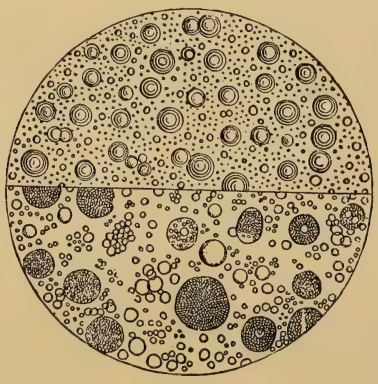

FIG. 35.

Cow's milk is subject to the same laws. A sickly cow gives unwholesome milk. Serious injury may be caused by the use of milk taken from a tuberculous cow, or one affected by "foot and mouth disease."

224. *Reaction.*—With red litmus paper test human milk; it turns blue, showing alkalinity. Cow's milk is

usually alkaline or neutral when fresh, though sometimes acid, and occasionally a sample will be found that is amphoteric—*i. e.*, reddens blue litmus paper, and turns red litmus paper blue.

225. *Quantity.*—The average daily quantity secreted by a woman is one liter, or two pints.

226. *Spontaneous Change.*—Let milk stand for several days in a warm place; it coagulates and "*sours*"—*i. e.*, turns acid. At the same time it would be advisable to conduct experiments (§§ 227, 228, 229).

This change is due to the conversion of the milk sugar (lactose) into lactic acid by a minute organism, the Bacterium lactis. The casein is normally held in solution by alkaline phosphates. Acids change the reaction and thus cause the casein to be precipitated.

227. To half a test-tube of diluted milk add a drop or two of hydrochloric or acetic acid and gently warm it; an abundant precipitate falls. This precipitate is the curdled casein, carrying with it most of the fat.

According to recent studies casein exists in solution as caseinogen, a form of union with calcium phosphate. The phosphate being removed by acids, the insoluble casein is precipitated.

PRESERVATION OF MILK.

228. *Refrigeration.*—Having tested with litmus paper, put a sample of fresh milk in a refrigerator kept at or below 50° F. (10° C.). After several days examine it again with litmus paper. The reaction will be but little changed, the milk having been kept sweet and uncurdled.

In spite of the temperature the lactic ferment will in time develop to such a degree as to impair the digestibility of cow's milk used for infants' food. While the growth of bacteria is checked by cold, the specific forms that cause disease are not destroyed by it.

229. *Sterilization.*—Put some fresh milk in a test-tube and boil it for from fifteen to twenty minutes. A scum will form, due to coagulation of the lact-albumin and globulin. Close it with a tight cork or a plug of cotton, and stand it aside for several days. It will keep sweet and uncurdled, owing to the death of the bacteria which cause lactic fermentation. At the same time, the fats, sugars, casein, and albumin are altered in such a way as to make boiled milk less digestible and nourishing than raw milk.

Cow's milk delivered in cities contains numbers of bacteria. It is usually twenty-four or forty-eight hours old before it is delivered, and in that time the bacteria have a chance to multiply rapidly. Most varieties of these microbes are entirely harmless, as is proved by the fact that mankind has been consuming milk for ages without injury from them. Without doubt *cholera*, *diphtheria*, *scarlatina*, and *typhoid fever* have been communicated by milk containing the specific germs or bacteria of these diseases. It is probable that *tuberculosis* can be conveyed in the same medium, the bacillus of tubercle having been found in the milk of tuberculous cows. The danger of its distribution is materially lessened by the circumstance that this germ can not multiply in milk. A small portion of tuberculous milk is simply diluted by being mixed with a large quantity that is not tuberculous, and thus the num-

ber of bacilli taken by any individual is relatively small. Experiment shows that this particular germ must be introduced in such numbers at a time, in order to cause disease, that the chances are against infection by it through ordinary mixed city milk.

Other bacteria produce certain intestinal troubles, such as *summer diarrhea* and *cholera infantum*. They have the power of multiplying in milk and developing *ptomains* or *toxalbumins*, which poison the stomach and intestines directly. A highly poisonous substance called *diazobenzene* or "tyrotoxicon" is sometimes developed in milk used in various foods, such as ice-cream. These poisons are not present in fresh milk, which is therefore harmless so far as *summer diarrhea* is concerned. Their production is prevented by boiling the milk when fresh. At the same time the high heat of 212° F. (100° C.) injures the milk as an assimilable food, and when it is convenient a method of lower heating known as Pasteurization is preferable.

230. *Pasteurization.*—Put some fresh milk in a test-tube or clean glass bottle, closing the neck with a plug of cotton. Stand the tube or bottle in a vessel of water, and heat the water to 160° F. for a few minutes, carefully observing the temperature by an immersed thermometer. Set is aside, stopped with the plug of cotton, and it will be found to keep for twenty-four hours at least.

Milk heated above 167° F. (75° C.) is so changed that children fed on it exclusively do not thrive. Adults find it less palatable and less digestible, the curds forming in whole masses instead of in flakes. At and below 167° F. (75° C.) it does not lose the taste of fresh milk nor be-

come less digestible. At this temperature and as low as 150° F. the matured disease germs are killed and the spores so much weakened in vitality that all liability to cause intestinal disorder is removed if the milk is used within twenty-four hours of the treatment. It must be noted that if the milk is not tolerably fresh, poisons may have become developed already. Pasteurizing will not destroy the toxalbumins or dissolved poisons when once produced, nor render stale milk harmless.

231. *Pasteurizing Apparatus.*—More or less elaborate apparatus, such as the Arnold sterilizer, can be purchased; but the following form is easily constructed, and the method simple in operation and sure. Take a pail made of tin or iron or copper, and have a tinner cut a disc of tin a little smaller than the bottom of the pail, with some strips of metal left radiating from the disc, which, when turned back, will serve as legs half an inch high; or insert a pie pan that has had holes punched in it. The disc must be perforated like a colander, so as to allow of free circulation of water when this false bottom is put into the pail. The bottle of milk is closed with a stopper, which has a glass thermometer fitted through a hole, or by a plug of cotton holding the thermometer with firm pressure. The bulb of the thermometer dips into the milk, while the scale can be read outside.

Set the bottle full of milk on the false bottom and fill the pail with water as high as the surface of the milk in the bottle. Heat the pail of water until the temperature of the milk reaches 155° F. Remove the bottle of milk and allow it to cool gradually, or the milk can be kept at 160° for ten minutes and then cooled rapidly.

When the thermometer is not at hand, at a pinch resort may be had to the following rough method. Put the fresh milk in clean glass bottles stopped with cotton. A basin containing several inches of water is placed on a slow fire and the bottles of milk set in it. After boiling the water for ten minutes, the milk, which has not boiled but only simmered, is removed and cooled before using.

232. *Milk Preserved by Chemicals.*—It is the custom of some dairymen supplying large cities in the summer time to add to their milk tasteless bactericides, such as formaldehyd 1 part to 50,000 (about two grains in the gallon) or boric acid 35 grains to the gallon. These preservatives prevent fermentation change in the milk for several days and do less harm to the person drinking the milk than would the bacteria that spoil the milk when kept too long.

233. *To Detect Formaldehyd.*—Put in a test-tube one inch of concentrated sulphuric acid and a drop of solution of ferric chlorid. Upon this float a mixture of milk and water equal parts. At the zone of contact pure milk is greenish, but formaldehyd milk is blue or purple.

234. *To Detect Borax or Boric Acid.*—In a porcelain dish, mix milk 1 drop; hydrochloric acid 2 drops; and saturated tincture of turmeric 2 drops. Dry on a water-bath, cool, add a drop of ammonia by a glass rod. Borax gives a blue color changing to green.

235. *Modified Milk.*—To make cow's milk more like human milk in composition it is modified artificially by dilution with water or lime-water, adding cream, and milk sugar. The proportions vary in accordance with the age of the child but an average mixture would contain cow's milk 2 fluidounces, cream 3 fluidounces, water 10

fluidounces, lime-water 1 fluidounce and milk sugar four drachms. For more exact directions consult works on infant feeding.

236. *Milk Sugar and Salts.*—Having filtered the whey from the acid curd (§ 238), test the whey with Fehling's solution. The milk sugar reduces the cupric oxid. To another portion add magnesia mixture; the phosphates are precipitated. To another portion add silver nitrate; the chlorids are precipitated, insoluble in nitric acid.

237. *Butter.*—To a test-tube one-third full of milk add half its volume of potassium hydrate and half of ether; shake the mixture and stand in a warm place. The milk clears up, and the butter dissolved in the ether floats at the top. On separating the ethereal layer and evaporating it, a residue of butter is left.

In cow's milk ether will not dissolve the fat globules unless they are liberated by dissolving their envelopes with acetic acid or caustic potash or soda. With human milk it suffices to agitate with ether alone. In ordinary churning the envelopes of casein are ruptured mechanically, and the fat globules cohere in large masses of butter. This process does not separate all the fat as butter. The residue, called "buttermilk," still contains about one per cent. of fat.

238. *Pepsin Curd.*—Into a test-tube about one-third full of milk put a few drops of neutral essence of pepsin (Fairchild's). Mix gently, warm to the temperature of the body, and keep at 104° F. (40° C.). A solid *curd* forms in ten or twelve minutes, so that the tube can be inverted without losing the milk. In a short time a *whey* separates from the clot.

239. *Junket, or Curds and Whey.*—To make this delicacy for invalids, take one-half pint of fresh milk, heated as hot as can be agreeably borne by the mouth, add one teaspoonful of rennet, or essence of pepsin (Fairchild's), and stir just enough to mix. Let it stand until firmly curdled; may be served plain or with sugar and grated nutmeg.

An egg beaten to a froth and sweetened with two teaspoonfuls of sugar may previously be added to the half-pint of milk, forming a highly nutritious and smooth jelly. The essence will curdle milk with egg as readily as plain milk.

240. *Whey.*—Curdle warm milk with the pepsin essence as above directed; then beat up with a fork until the curd is finely divided; now strain, and the whey is ready for use.

Whey is useful in diet for the sick. It is frequently resorted to as a food for infants to tide over periods of indigestion.

241. To the curd obtained (§ 238) add a few drops of dilute hydrochloric acid, so as to make with the pepsin an artificial gastric juice, and set aside at 104° F. for two or three hours. The curd is digested and gradually dissolves to make a yellowish fluid with the peculiar odor and bitter taste of "peptonized milk."

If the milk is previously boiled, or if the rennet is boiled, the ferment will not work.

It acts best at a temperature that can be borne in the mouth—*i. e.*, about 104° F.

242. *Specific Gravity.*—Shake well a specimen of fresh or "whole milk," and pour into a cylindric glass vessel

until two-thirds full. Slowly immerse a hydrometer; it will record at the surface somewhere between 1029 and 1035 (Fig. 36).

243. The specific gravity of dairy milk, the product of a number of cows, should never fall below 1029. When lower than this, it is usually due to adulteration with water; but very rarely the low density is due to excess of cream in very rich milk.

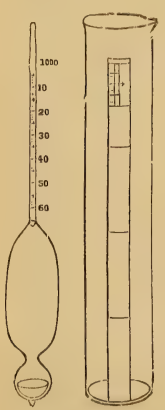

FIG. 36.
Hydrometer.— (Starr.) Creamometer.— (Starr.)

244. Let the milk stand for twenty-four hours, "skim," and remove the "cream" which has risen to the surface. Now take the specific gravity: it will range between 1032 and 1040.

The fat globules of the cream in "whole milk" lower the gravity, because fat is lighter than water. "Skim milk" is heavier than fresh or "whole milk," but this difference may be removed by adding water, which is also lighter than milk.

245. *Correction for Temperature.*—As cold milk is heavier than warm milk—*i. e.*, has a higher specific gravity—a correction for temperature is necessary. For ordinary hydrometers, adjusted at 60° F., an approximately correct specific gravity is obtained by subtracting one from the hydrometer reading for each 10° F. *below* 60° F., and by adding one to the reading for each 10° F. above 60° F. For variations of less than 10° F., the proportionate fraction of one must be added or subtracted, as the case may be.

MILK TESTING.

246. (*a*) *Take the specific gravity with a hydrometer and correct for Temperature.* (Fig. 36.)

Any milk that stands above 1033 (33 of the scale) is almost sure to be skimmed, while that falling below 29 of the scale is nearly always watered. The only exception to this rule is in the case of a very rich milk containing an excess of cream. This excess would be apparent to the unaided eye by the consistency and the "creamy" appearance. There is a trick known to the milk trade by which the indications yielded by the specific gravity may prove fallacious. Some of the cream is skimmed off of fresh milk with a specific gravity, of about 1030, leaving a milk of higher specific gravity, about 1035. By adding water in proper amounts the specific gravity may be lowered again to that of the original whole milk. The trained eye may detect a change in color and consistency, but we can not depend on the *creamometer* (a graduated cylinder by which we measure the layer of cream that rises in a certain time). A milk that has been watered will in a few hours separate a thicker layer of cream than would the same amount of the unwatered. The separation takes place with greater rapidity in the diluted than in the whole milk. The *lactometer* of the New York Board of Health is a hydrometer which has a scale on which 100° stands for a specific gravity of 1029 (the minimum density of pure milk), while 0° stands for the specific gravity of water, and 120° for 1034, the maximum range of pure milk. On this instrument 1° is read as one per cent. of milk in the sample.

247. (*b*) *Note the Color and Consistency.*—For clinical study of mother's milk or ordinary sanitary testing of cow's milk Feser's lactoscope gives valuable results.

A rich milk looks like cream, and is thicker than a skimmed milk. If the color is bluish or less creamy, and the fluid less opaque, while the specific gravity is less than 1029 or under 100° of the New York lactometer, then it is safe to assume that the milk has been watered. A pure rich milk is perceptibly more opaque than a skimmed or watered sample. The opacity or white color of the milk is due to the suspended fat globules, and is proportionate to the number of them. By measuring this opacity an approximate estimate can be made of the percentage of fat. For making this estimate roughly, Heeren's pioscope may be used, but *Feser's lactoscope* (Fig. 37) is very convenient and more accurate. In the axis of a cylindric, clear, glass vessel, and at its lower part (A), is a smaller cylinder of white glass, marked with a few black lines. In testing with this instrument four cubic centimeters of milk are introduced with the graduated pipet; the black lines are entirely concealed. Pure water is gradually added, while shaking, until the milk clears up sufficiently to make the black lines distinctly visible; sharp definition of the lines is required, not simply a dim showing of them. By the graduation on the vessel the surface level of diluted milk can be read as percentage of fat in the original sample. The microscope having determined the absence of chalk, starch, or other suspended adulterants, a sample showing three per cent. and over is judged pure. Some rich Jersey milk shows six per cent. Any one experienced in its use will be accurate to within one-

fourth of one per cent. Having obtained the specific gravity by the lactometer, and the percentage of fat by the lactoscope, experiment shows that the proportion of total *solids* can be calculated by the formula of Hehner and Richmond given on page 158.

248. (*c*) *Measure the Amount of Cream.* (Fig. 36.)

For this purpose a creamometer or a 100 c.c. glass cylinder graduate may be used. Having mixed the milk thoroughly, a sample is poured into the vessel up to the highest mark. After standing in a cool place for twenty-four hours the depth of cream layer thrown up is read off. It ought to show from ten to twenty volumes in the hundred. The average sample would be twelve per cent. If the cream forms twenty per cent. of the column, the sample would probably also show a low specific gravity. The accuracy of this test is affected by the length of time since milking, by the amount of previous agitation of the milk, by the fact that dilution causes a more rapid separation of the cream, by the temperature, and other variable conditions. It may serve a useful purpose when taken in consideration with other observations.

FIG. 37.—Feser's Lactoscope.—(Queen.)

Less than ten per cent. of cream in a milk of specific gravity above 1033 *denotes skimming.*

Less than twenty per cent. of cream, if joined to a specific gravity less than 1029, *indicates watering.*

DETERMINATION OF WATER, SOLIDS, ASH, AND FAT.

249. MILK STANDARDS.—In a number of States a chemic standard of purity has been fixed by law. All samples of cow's milk falling short of this are assumed to be adulterated. By the United States Treasury Department, it should contain by weight not less than 13 per cent. of total solids and not less than 3.5 per cent. of fat. In Philadelphia the ordinance of 1890 requires not less than 12 per cent. of solids nor less than 3.5 per cent. of fat. In the State of Pennsylvania it must contain not less than 12.5 per cent. of solids and not less than 3 per cent. of fat. In the States of New York and New Jersey it should contain not less than 12 per cent. of solids, nor less than 3 per cent. of fat.

250. *Total Solids.*—Into a tared dish, preferably of platinum,—though a watch-glass will answer,—five grams of milk are weighed or five cubic centimeters measured. The dish is then exposed to the heat of a water-bath for three hours. As evaporation is nearly done, it is now put into a water oven, and at intervals weighed until it ceases to lose weight. This constant weight, less the weight of the capsule, gives the *total solids*. The difference between the five grams and the constant weight of the dry solids represents the *water*. By carefully incinerating the solids to a grayish-white color the *ash* or inorganic salts are determined. In pure milk the amount ranges from 0.7 to 0.8 per cent. A watered milk will show a reduced amount both of solids and of ash.

251. *Fat by the Werner-Schmid Process.*—This is an easy, rapid and quite accurate method. Into a long test-tube with a capacity of 50 c.c., and graduated to show cubic centimeters in tens, measure 10 c.c. of milk and 10 c.c. of strong hydrochloric acid. (A large common test-tube can be used, and the measurement made by pipets or other graduated glasses.) The mixture of acid and milk is boiled one and one-half minutes, or the tube may be corked and heated in a water bath for five or ten minutes, until the liquid turns a deep brown, but not black. Having cooled the tube and its contents in running water, 30 c.c. of well washed ether must be added, the tube corked, the mixture well shaken, and finally stood aside. When the line of separation between ether and acid is distinct, a wash-bottle cork stopper with its tubes, is substituted for the plain stopper. (See Fig. 38.)

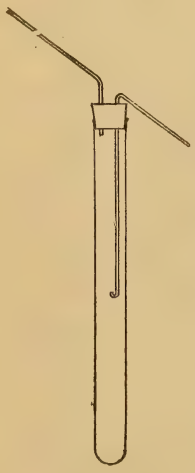

FIG. 38.

The lower end of the exit tube has a short curve, which is adjusted so that its opening is just above the line of separation. A weighed flask or beaker is held so as to receive the ethereal layer when it is blown out by the lips at the upper tube. In succession two additional portions of washed ether, 10 c.c. each, are shaken with the acid and blown out into the weighed flask. The ether is then distilled off or evaporated, and the fat residue dried in a water oven and weighed. It is the amount contained in 10 c.c. of milk.

252. *Fat.*—To determine the butter by the gravimetric method, ten grams of milk are weighed into a tared dish containing a weighed amount of dry sand. The milk is evaporated on a water bath and last on a water oven, with constant stirring. The residue is washed a number of times with warm ether or petroleum naphtha of 70° Baumè, and the washings passed through a small filter. The filtrates are all received in a tared beaker and carefully evaporated to a constant weight. The residue is *fat.* This subtracted from the amount of total solids gives the *solids not fat.*

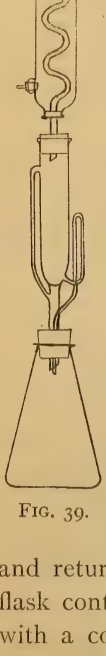

FIG. 39.

253. *Fat by Adam's Method.*—This is the standard process in use by official chemists who have well-equipped laboratories. The milk is absorbed by strips of pure, fat-free paper, which distributes the milk fat in a thin layer. The coiled strip is dried in a water oven, and then placed in the middle chamber of a Soxhlet extractor. (Fig. 39.) The tared flask containing 75 c.c. of ether is heated on a water-bath.

Ether vapor condenses in the upper apparatus, flows back upon the coil of paper, and returns to the flask. After ten such washings, the flask containing the ether is detached and connected with a condenser. After distillation the fat residue is dried in an air oven, cooled, and weighed.

254. *The Centrifugal Method.*—To obtain satisfactory separation of fat from either breast or cow's milk, the graduated milk bottle (Fig. 40) may be used with any

medical centrifuge. It gives results accurate to within 1/5 of 1 per cent. of fat.

Fig. 40.
Milk Bottle.

In the usual apparatus two pipets are supplied with the bottles, one of five cubic centimeters' capacity, marked "milk"; the other, holding one cubic centimeter up to a mark on the lower stem, for introducing the alcoholic solution.

To determine fat by this method the sample should be taken from the middle portion of the nursing or milking—as the first milk is poorer, and the last richer, than the average. Five cubic centimeters of the sample are introduced into the milk bottle by means of the pipet marked "milk"; 1 c.c. of alcoholic solution (No. 1) is added and shaken by hand. Then, by means of large pipet, solution No. 2 is added little by little, with shaking, until the bottle is filled to the brim. It is then placed in the centrifuge and whirled from one to two minutes, when the fat rises to the top in a clear yellowish layer, and can be read off in direct percentages. A few drops of water may be added if necessary to bring the fat to the zero point; a whirl of the centrifuge brings the fat above the water. If the milk should be richer than 5 per cent. add 5 c.c. of water to 5 c.c. of milk, mix thoroughly, take 5 c.c. for analysis, and multiply the result by 2. For cream, add 20 c.c. of water to 5 c.c. cream,

mix, take 5 c.c. for analysis, and multiply the result by 5.

Solution No. 1 consists of

Amyl alcohol,	37 parts by volume.
Wood alcohol,	13 parts by volume.
Hydrochloric acid,	50 parts by volume.

This solution can be kept some weeks. If it turns dark, a fresh mixture must be made.

Solution No. 2 consists of

Sulphuric acid; specific gravity, 1.832.

255. *Formulas of Hehner and Richmond.*—These are used to facilitate analysis of mother's milk for clinical purposes, and sometimes in sanitary sorting of cow's milk, when rigid accuracy is not required but time must be saved. By these formulas any one of the three data—specific gravity, fat, and total solids—can be calculated when the other two are known. Having ascertained the specific gravity with the *lactometer* and the fat with *Feser's lactoscope* (see § 247), or by the *Werner-Schmid* process, or by the centrifuge the analyst can be spared the tedious process of determining the total solids by evaporation. When the fat and the specific gravity are known, the formula for estimating total solids is

$$T = \frac{F + 0.2186\ G,}{0.859}$$

in which F stands for percentage of fat, T the percentage of total solids, and G the specific gravity expressed in the last two units and any decimal; thus, if the specific gravity is 1028.5, then G stands for 28.5. For example,

if a specimen of milk had a specific gravity of 1030, and the percentage of fat was 4, then—

$$\text{Total solids} = \frac{4 + (0.2186 \times 30)}{0.859} = 12.3 \text{ per cent.}$$

When the specific gravity and the total solids are known, then for ordinary whole milk

$$F = 0.859\ T - 0.2186\ G.$$

For poor skim milk there is a possible error of small amount; and when $\frac{G}{T}$ exceeds 2.5, some change is necessary;

$$F = 0.859\ T - 0.2186 - 0.05\ (\tfrac{G}{T} - 2.5).$$

Having determined the total solids by evaporating to dryness 2 or 3 c.c. of milk in a watch-glass, the *solids not fat* will be the remainder after subtracting the fat from the total solids.

256. *Watered Milk.*—To percentage of pure milk in a suspected sample may be calculated by the following formula using the figures of the Philadelphia standard—*i. e.*, 12 per cent. of total solids, 3.5 per cent. of fat, and 8.5 per cent. of solids not fat. The solids not fat may have been learned by the observation or calculations stated before.

Legal standard.		Observed.			Whole milk.	
8.5	:	solids not fat	::	100	:	x.

In this equation x represents the amount of whole milk used in making 100 parts of the mixture, and 8.5 is the legal per cent. of solids not fat. Thus, a sample when analyzed by lactoscope or centrifuge and the data calcu-

lated by the above formula, (p. 159) yielded 7 per cent. of solids not fat; what was the proportion of pure milk?

$$8.5 : 7 :: 100 : x = 82.35.$$

The sample contained 82.35 per cent. of milk and 17.65 per cent. of water.

257. *Skimmed Milk.*—To estimate the percentage of fat taken away by skimming the following formula may be used, S standing for solids not fat and F for fat found by lactoscope or other means.

$$3/9 \times (S - F) = x.$$

Thus if a sample showed 9 per cent. of solids not fat and 1.5 per cent. of fat found then

$$3/9 \times (9 - 1.5) = x = 2.66.$$

The milk had been deprived of 2.66 per cent. of fat.

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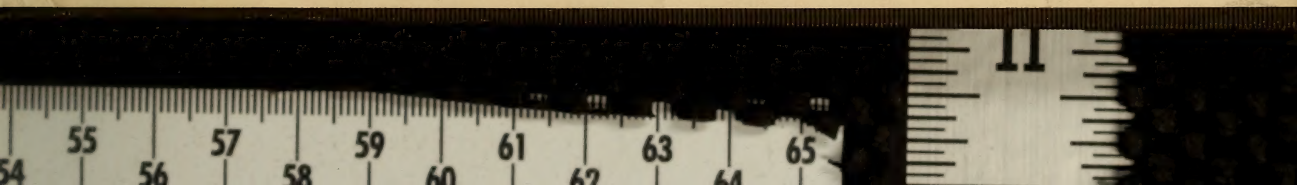
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